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PRESBYTERIAN-ST. LUKE'S HOSPITAL

MEDICAL BULLETIN

VOL. 1 NO. 1 JUNE 1, 1962

Primary Pharyngeal Malignant Lymphomas

Post-Irradiation Hyperlipemia

Abdominal Aortic Graft Complications

Uptake of Tritiated Thymidine into Human Tumor DNA

Vitreous Hemorrhage—A Retinal Tear Until Proven Otherwise

Anemia in Rheumatoid Arthritis

The Neuraminidases of Influenza Virus

Abstracts and Reviews

THE PRESBYTERIAN-ST. LUKE'S HOSPITAL MEDICAL BULLETIN

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The Presbyterian-St. Luke's Hospital Medical Bulletin is published quarterly by the staff of the Presbyterian-St. Luke's Hospital and its Alumni Foundation. It will be sent to those interested on request.

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Bulletin
1753 W. Congress Parkway
Chicago 12, Illinois

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MALIGNANT LYMPHOMAS PRESENTING AS PRIMARY PHARYNGEAL LESIONS

Stanton A. Friedberg, M.D.

George M. Hass, M.D.

A tumor of the nasopharynx or pharynx may represent the initial lesion in certain lymphoid disorders. Although the exact classification of these diseases has long been controversial, the tendency now is to designate them under the term "malignant lymphomas." Included in this grouping are Hodgkin's disease, giant follicular lymphadenopathy, lymphosarcoma of various types and lymphatic leukemia.[1]

We have studied 18 patients whose pharyngeal lesions produced the first symptoms of their systemic disorder. At the time of original examination there was no evidence of generalized disease.

GROSS APPEARANCE

When first seen, the following anatomical sites were involved: (Figs. 1, 2 and 3).

Nasopharynx	7
Pharyngeal Wall	4
Tonsil	7

In 4 patients, continuity existed between the nasopharyngeal mass and the swelling of the pharyngeal wall. Inferences as to the primary area of involvement were drawn from the chronology of symptoms and the local examination.

The nasopharyngeal masses, for the most part, were originally described as "abnormal-appearing lymphoid tissue." In the two youngest patients *(aged 10 and 18)* previous adenoidectomies, performed elsewhere for nasal obstruction and deafness, had been followed by rapid recurrence of lymphoid tissue in the nasopharynx. Among older individuals the presence of adenoid tissue was usually not suspected. When seen by trained examiners it was immediately regarded with suspicion *(Fig. 2)*.

A smooth unilateral swelling with intact overlying mucosa was the usual physical finding with pharyngeal wall involvement *(Fig. 3)*.

Unilateral tonsillar hypertrophy without ulceration occurred in all patients in this category save one (with Hodgkin's disease) and in two instances this enlargement was of such degree as to cause severe impairment of respiratory and swallowing function *(Fig. 1)*. "Peritonsillar abscess" was the referring physician's diagnosis in three patients.

In the entire series there were seven patients in whom one or more cervical nodes were palpable. In the majority,

From the Department of Otolaryngology and Broncho-Esophagology and the Division of Pathology.

Fig. 1.—Large non-ulcerated mass occupying left tonsil fossa, almost entirely occluding oropharynx Arrow points to only remaining communication between mouth and pharynx. Malignant lymphoma lymphocytic type.

Fig. 2.—Pharyngeal mass behind posterior tonsillar pillar on right side. The tumor extended from the epipharynx to the tip of the epiglottis. Malignant lymphoma, lymphocytic type.

Fig. 3.—Mirror view of nasopharynx showing tumor mass of sufficient size to occlude both posterior choanae and produce nasal obstruction. Malignant lymphoma, lymphoblastic type.

excisional biopsy was performed and revealed changes related to those seen in the primary lesion.

In only one patient was death the result of local extension of the original lesion. In this individual an error in establishing the correct diagnosis probably accounted for intracranial spread of a reticulum cell sarcoma of the nasopharynx.

RESUMÉ OF CASES

A general statement regarding microscopic findings in these tumors is impossible because of the variability of cellular components in any given area and the multiplicity of tissue changes which may become evident as the disease manifests itself in other parts of the body. It is convenient to attempt a classification but we have been repeatedly impressed with the lack of any sharp distinction between the different types of malignant lymphoma.[2] One is led to suspect that the forms described represent variants of the same process and that multicentric foci of origin are implicated in the pathogenesis.

SUMMARY OF TYPES OF MALIGNANT LYMPHOMA

18 patients

Giant Follicle	2
Lymphocytic	2
Hodgkin's	3
Lymphoblastic	4
Reticulum cell	7

Giant Follicle (Brill Symmers) Lymphadenopathy

One patient, a 19 year old female, had repeated adenoidectomies followed by irradiation therapy to the nasopharynx and neck. She has remained free of disease for 10 years. The distinction between inflammatory hyperplasia and tumor formation may be extremely difficult, as it was in this case.

The second patient, a 42 year old male, has had no local or systemic re-

currence for 12 months following excision of a tonsillar mass plus irradiation therapy.

Lymphocytic Type

One patient, a 57 year old female, presented evidence of systemic involvement 10 months after establishment of diagnosis on pharyngeal biopsy. She succumbed in 2 years as a result of suppression of bone marrow function incidental to attempts to control widespread lymphomatous disease.

Another individual, a 53 year old male with a tonsillar tumor of similar histology, was well for 8 years until recently when the appearance of pulmonary and axillary nodules has indicated recrudescence of disease.

Prognosis based on similarity of cell types appears no more dependable than predictions based upon age, sex or postulated degree of malignancy of a particular tumor.

Hodgkin's Type

None of the 3 patients in this group is living *(Fig. 4)*. Two survived 6 and 8 years respectively. Interestingly enough, the original diagnoses of lymphocytic and lymphoblastic lymphoma were changed to Hodgkin's disease as new foci of disease appeared with accompanying alterations in the histologic picture. The interrelationship of Hodgkin's disease and other lymphatic neoplasms has been described by Custer and Bernhard.[3]

Lymphoblastic Type

Two of the patients of the four in this category have died of systemic disease. The third, a 59 year old female with an original nasopharyngeal mass, has recently shown evidence of disseminated disease after an initial remission period of one year. The fourth patient, a 60 year old male with a tonsillar tumor, has undergone surgery and irradiation too recently to permit any prognostic attempts.

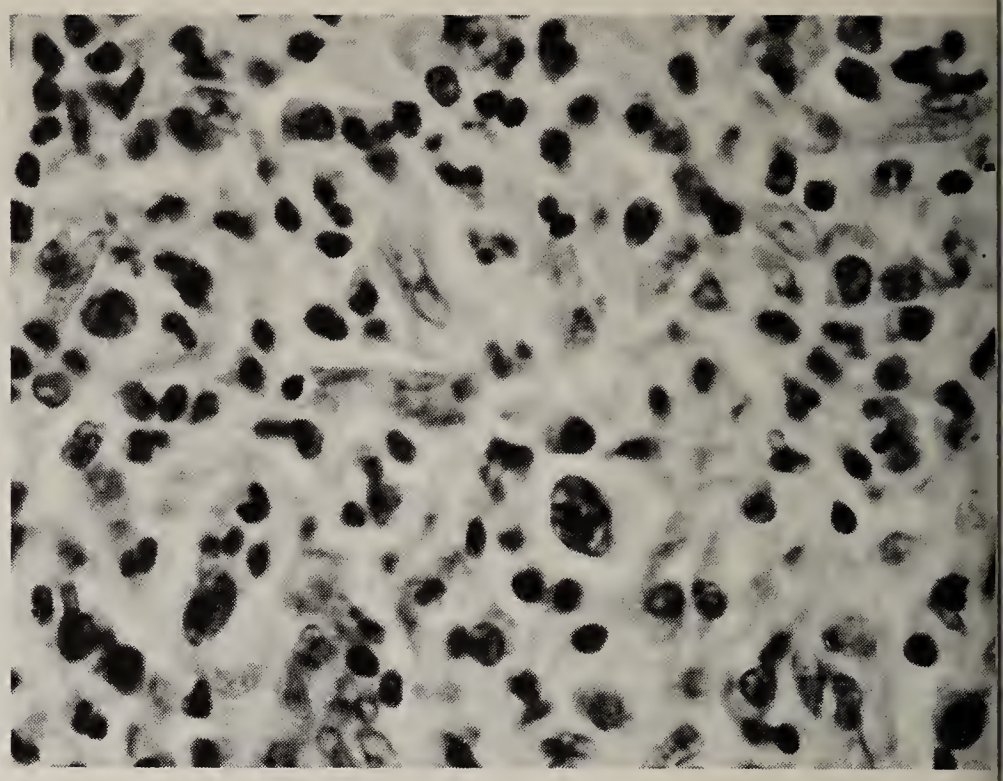

Fig. 4.—A, biopsy section of tonsil, showing absence of organized lymphoid tissue. The preponderant cell type is the eosinophile. Hodgkin's disease of the tonsil.

Reticulum Cell Type

This group of 11 patients is the largest in the series. All but one of the patients have expired. One female, aged 44 when the diagnosis was established, experienced a 5 year remission but survived for another 5 years *(Fig. 5)*. The only living patient has had an unusual clinical course. This man, age 40, had an extensive pharyngeal mass diagnosed as reticulum cell sarcoma in 1951 and

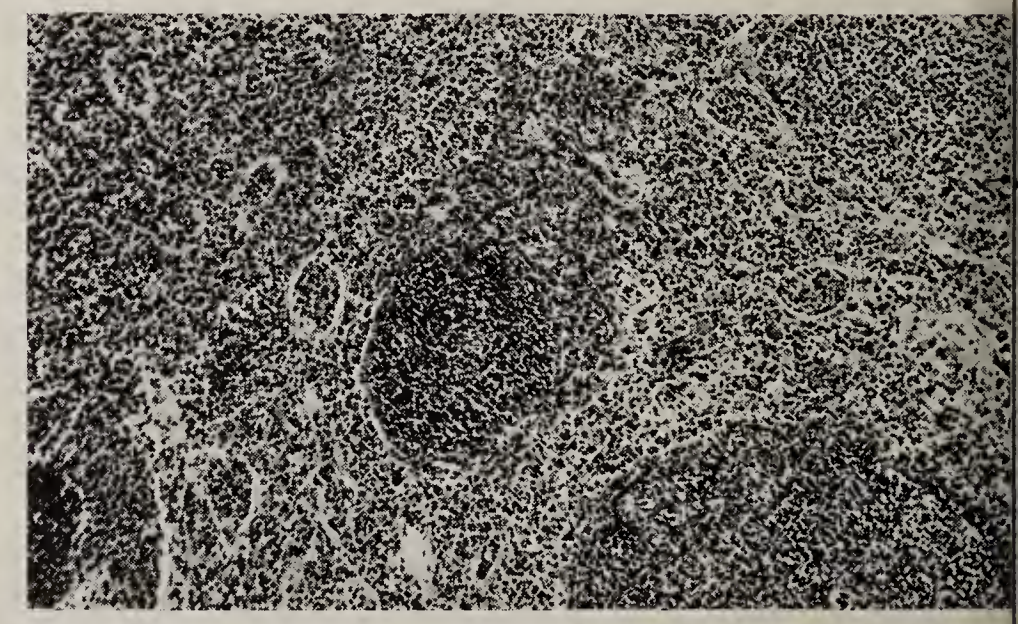

Fig. 5.—Biopsy section of the nasopharynx, showing many immature reticulum cells in the interfollicular areas and in the lymphatic sinuses. Reticulum cell sarcoma.

treated by irradiation. There was complete regression of the tumor. Six months later an intestinal obstruction necessitated bowel resection, at which time widespread involvement of retroperitoneal lymph nodes was noted. The obstructing mass and lymph nodes were histologically characteristic of reticulum cell sarcoma. Irradiation therapy was again administered. He has remained completely symptom free for the past 10 years. This case is the one exception to the statement that the only prognostic sign of any significance in these cases seems to be the initial period of remission.

In following these patients clinically and reviewing tissues from the original sites, tissue from areas of new involvement and material made available at post mortem examination, we have come to agree with Gall and Mallory that nothing in the histologic character of an individual lesion will permit a reliable forecast as to the distribution of the process in other parts of the body.[4] We have also been impressed with the overlapping microscopic features in many cases. For example, in tissues from one patient we were able to note characteristics of reticulum cell sarcoma, lymphoblastic sarcoma and Hodgkin's disease. As mentioned, original diagnosis were subsequently changed to Hodgkin's disease in two patients. Actually, these difficulties in diagnosis are often more apparent to the pathologist than to the clinician.

We have become aware of the fallacy of depending upon cell type as a basis for predictability of future behavior of malignant lymphomas. There is a question as to whether the histologic diagnosis made by the pathologist has any significance other than the establishment of the diagnosis of malignant lymphoma.[5]

In none of the patients in this series were the white blood counts or differential blood smears of any diagnostic significance in relation to the diagnosis of the primary disease.

SYMPTOMATOLOGY

The symptoms which cause these patients to seek medical attention are frequently so commonplace that they may either be ignored or regarded too casually. Nasal obstruction and impaired hearing were the presenting complaints in those individuals with nasopharyngeal primary lesions. Throat discomfort of varying degree was universally reported. In some patients this was described as merely a sensation of fullness. Operations had been performed on the nose, nasopharynx and pharynx in some patients with only transitory benefit and antibiotics had been liberally administered.

Great difficulty in swallowing occurred in several patients whose tonsillar neoplasms filled the entire oropharynx. Masses of this size also interfered with the quality and resonance of the voice and in two cases produced severe respiratory obstruction.

Treatment of these areas by surgical excision plus irradiation was entirely successful in controlling the local disease in all cases but one. Consequently the symptoms of the primary lesion disappeared early and in many cases were forgotten as new foci or severe systemic involvement supervened.

DIFFERENTIAL DIAGNOSIS

The finding of any abnormal or recurrent mass of lymphoid tissue in the nasopharynx, of a smooth unilateral swelling of the lateral pharyngeal wall, or of a unilateral tonsillar hypertrophy, with or without ulceration, should arouse suspicion as to the possibility of lymphoma.

One should consider first the various inflammatory disorders affecting these regions, bearing in mind that the distinction between inflammatory lymphoid hyperplasia and actual neoplasm may occasionally be difficult to ascertain. Tuberculous retropharyngeal abscess arising from chronic tuberculosis of the cervical vertebrae may resemble lymphoma of the pharynx, but the diagnosis should be ap-

parent from roentgenograms. Tuberculosis of the nasopharynx, pharynx, or tonsillar region is seldom, if ever, seen independently of pulmonary tuberculosis. Acute inflammatory disease, such as pharyngomaxillary space infections and infections causing ulceration and exudation, can usually be eliminated on the basis of cultures and other laboratory data.

Various tumors arising in these areas may resemble lymphomas in their clinical appearance. Among these are the mixed tumors of salivary type developing in mucous glands of the pharyngeal and palatal mucosa, adenocarcinomas of similar origin, and lymphoepitheliomas. The last-mentioned type is composed of mixed epithelial and lymphoid elements and probably represents a form of transitional cell carcinoma. Not infrequently a painless firm cervical adenopathy may be the first and only symptom of this type of tumor.

Neurofibroma *(neurilemmoma, schwannoma)* of the cranial nerves, particularly the vagus, may produce a smooth tumor mass in the lateral pharyngeal wall, resembling lymphoma. Chordomas and carotid body tumors, though rare, must be kept in mind.

The greatest problems in diagnosis arise in attempts to differentiate the lymphoid disease themselves. The blood picture is nonspecific in patients with lymphoma, although as the disease advances, anemia invariably becomes evident. As Craver has pointed out, there are no clinical features which, without exception, will permit differentiation of tuberculous lymphadenitis, Hodgkin's disease, lymphosarcoma, aleukemic lymphatic leukemia, lymphoepithelioma, and even undifferentiated metastatic carcinoma in lymph nodes.[1] To this group might be added the plasmocytomas, which may be either single or multiple and are occasionally found in the areas under consideration.

Thus, one must rely entirely upon biopsy of the local tumor mass. If possible, a cervical lymph node should be removed for study at the same time *(Fig. 6)*.

Once the tissue is available, the various interpretations become a matter of importance and concern. We have noted how varying components of the lymphoid series may be simultaneously present in a given case or may make their appearance at different stages of the disease. Final judgment must, therefore, ultimately be placed upon continued study of the histopathological material and correlation of the resulting data with clinical manifestations.

Fig. 6.—Biopsy section of cervical lymph node with partial obliteration of follicular pattern. The outlines of a few follicles may be noted but the intervening neoplastic tissue predominates. Reticulum cell sarcoma.

TREATMENT

Irradiation therapy is the most effective form of treament for localized malignant lymphomas, even though it will rarely cure the disease. In this series of patients the primary lesions of the nasopharynx, pharynx and larynx were removed surgically for excisional biopsy purposes when indicated, or for the relief of mechanical obstruction. In all instances irradiation therapy was then employed and, with one exception, resulted in control of the local disease.

Basically, however, malignant lymphoma is a systemic disease and new foci invariably appear. This pattern is so typical that, should the patient remain free of disease, reappraisal of the original diagnosis is required.

Although surgical intervention may be indicated occasionally as new foci appear, reliance upon further irradiation or chemotherapy is the usual sequence.

Continuing weight loss and anemia may point to developing foci elsewhere in the body or, as occurred in some of the patients, to suppression of bone marrow function incidental to the therapy.

Various chemotherapeutic agents and steroid therapy were used with some measure of success for control of generalized lymphomatosis. In our recent experience, however, it has seemed that in many instances the use of chemotherapy may have shortened rather than prolonged the lives of some patients. In these patients, total elimination of the disease was achieved at the expense of undesirable action of the drugs upon non-neoplastic tissues. Consequently, extensive tissue alterations resulting from attempts to control disseminated disease were apparently responsible for the fatal outcome in some patients. Despite these adverse occurrences, chemotherapy probably offers the greatest potential for future therapy in this disease.[6]

SUMMARY

There were 18 patients in this series, *(10 males and 8 females)* and the age span ranged from 6 to 96 years. The data on all patients are available.

Twelve patients are deceased, two are living with evidence of disseminated disease and two are living and apparently symptom-free 10 years after initial treatment. Two patients were first seen just one year ago, too recently to warrant any conclusions as to prognosis.

The histologic type of malignant lymphoma had no particular prognostic significance except that the giant follicle type is alleged to have the best outlook.

The initial period of remission seemed to be the only significant sign relative to prognosis. The longest survival times *(with one exception)* occurred in patients whose initial period of remission ranged from 5 to 6 years.

Of the 6 patients who showed signs of generalized disease soon after diagnosis by pharyngeal biopsy, none lived more than two years.

There was marked variation in the behavior of malignant lymphomas from patient to patient. Hall and Olson have summarized the conclusions with which most writers agree:[5] "Some patients do not respond to any therapy and die within a few months. Many have remissions of a few months, respond less and less with future therapy, and die in from two to three years after the diagnosis is made. Some have a course similar to that of the latter group but remissions are longer, spread of the disease is slower, and they live five, ten or 15 years. In some cases these patients have a long remission from a single course of therapy and then show a rapid downhill course. Other long survivors have shorter single remissions but continue to respond to treatment for years. A few patients will remain apparently free of disease for periods of up to 25 years and then relapse and die of their disease. In some instances, especially with visceral tumors of the lymphosarcoma group, there appears to be a complete cure."

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Appreciation is expressed to Dr. L. J. Wallner for the clinical photographs.

POST—IRRADIATION HYPERLIPEMIA

Maurice L. Bogdonoff, M.D.

Numerous after-effects of external whole-body or segmental irradiation have been observed in experimental animals and in humans. Many of these are obvious and well known. That these effects are in some way time-dose-volume-organ dependent has been well established, though the details of these parameters remain to be evaluated critically. Studies have been carried out to assess some of the metabolic changes produced in rabbits and dogs by external irradiation, particularly in moderate to low dose ranges.

In 1949, Rosenthal[1] described opalescence of serum in irradiated rabbits and concluded that the degree of opalescence was correlated with the incidence of death in those animals. This correlation was confirmed by Hays and Hewitt[2]. Goldwater and Entenman[3] demonstrated that the lipoprotein levels were more closely correlated with the seriousness of post-irradiation injury than were leukocyte or erythrocyte counts, sedimentation rates or hematocrit. An Atomic Energy Commission group[4] reached similar conclusions concerning heavily irradiated dogs. These prior studies were done following whole-body irradiation at or beyond the LD 50 level. In some instances, the exposure doses were greatly in excess of the LD 50 level.

METHODS: Male New Zealand rabbits, weighing 2.25 to 4.3 kg. were separately caged and fed standard Purina rabbit chow and water. Male and female mongrel dogs, weighing 8 to 15 kg. were fed standard laboratory chow and water and were individually caged. The rabbits were irradiated in a wooden container with a head-holder; the dogs, in a cardboard container with strap-holders and appropriate lead shielding. The x-ray unit employed was operated at 250 kv, 20 ma, h.v.l. of 1.6 mm. of Cu, and a TSD of 50 cm. or 100 cm. The dose rate was about 45 r per minute for segmental irradiation and 10 r per minute for whole-body irradiation. Dosimetry was carried out with a Presdwood phantom and a Victoreen r meter. All exposures were single ones. Blood samples were obtained from the marginal vein of the rabbit ear and from the external jugular or anterior leg vein of the dog. During the first three days following exposure to x-rays, samples in both the rabbit and dog groups were obtained every eight hours. The animals were kept for about one year and then used for other purposes. Determinations of enzyme activity, serum lipids, fasting blood sugar, blood urea nitrogen, and serum electrophoresis were carried out by routine methods in the laboratories of Presbyterian-St. Luke's Hospital. Phospholipids were calculated from lipid phosphorus, and neutral fat was calculated by subtracting the sum of the values for cholesterol fatty acids and phospholipid fatty acids from the total fatty acid. In each test group, there were at least 6 animals.

RESULTS: Initially the studies were done on rabbits. Pre-irradiation and multiple post-irradiation serum values for enzymes and lipids were determined.

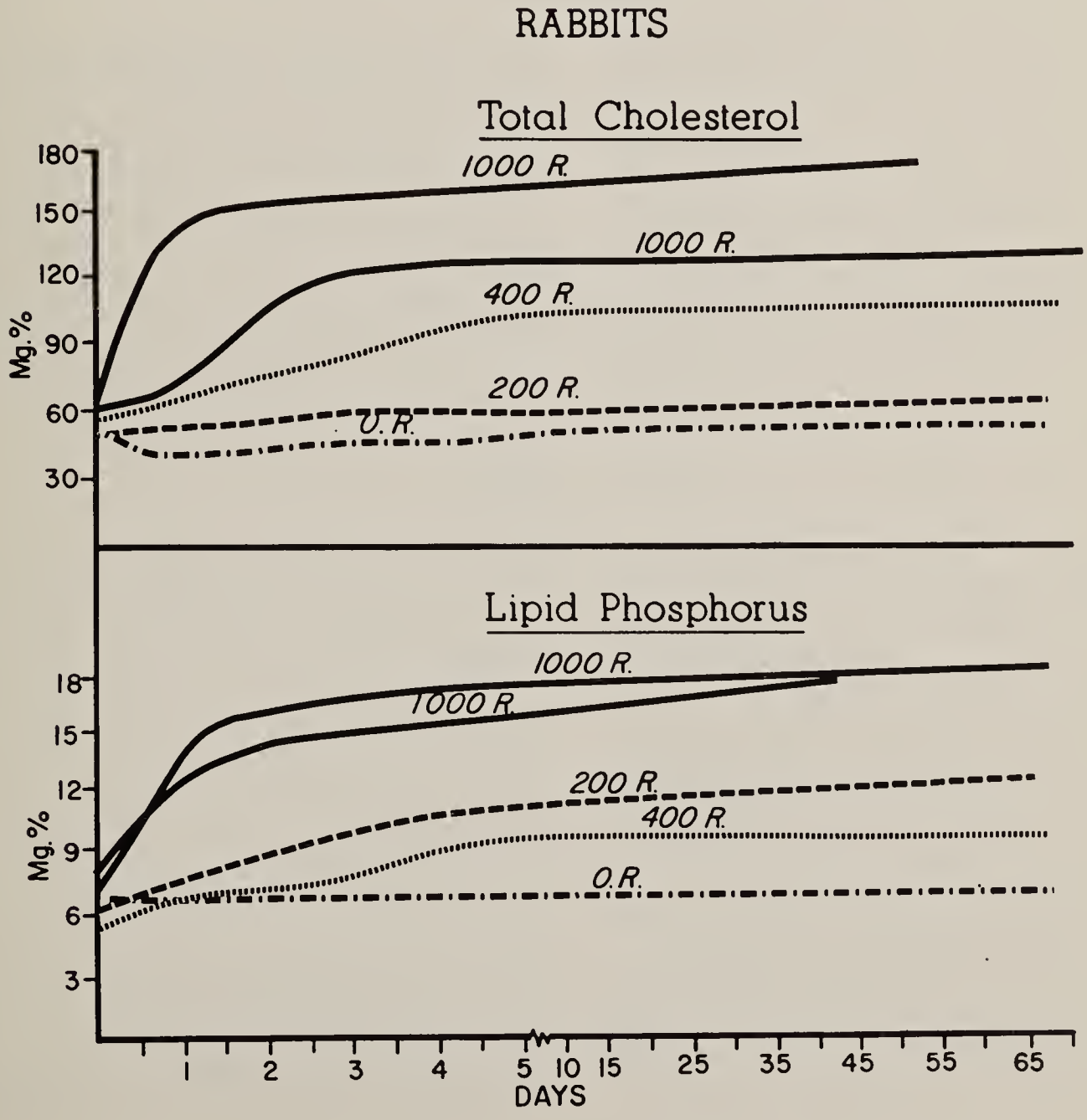
From the Division of Radiology and Nuclear Medicine

A portion of this study was supported by Presbyterian-St. Luke's Hospital Grant No. 48-40-37008.

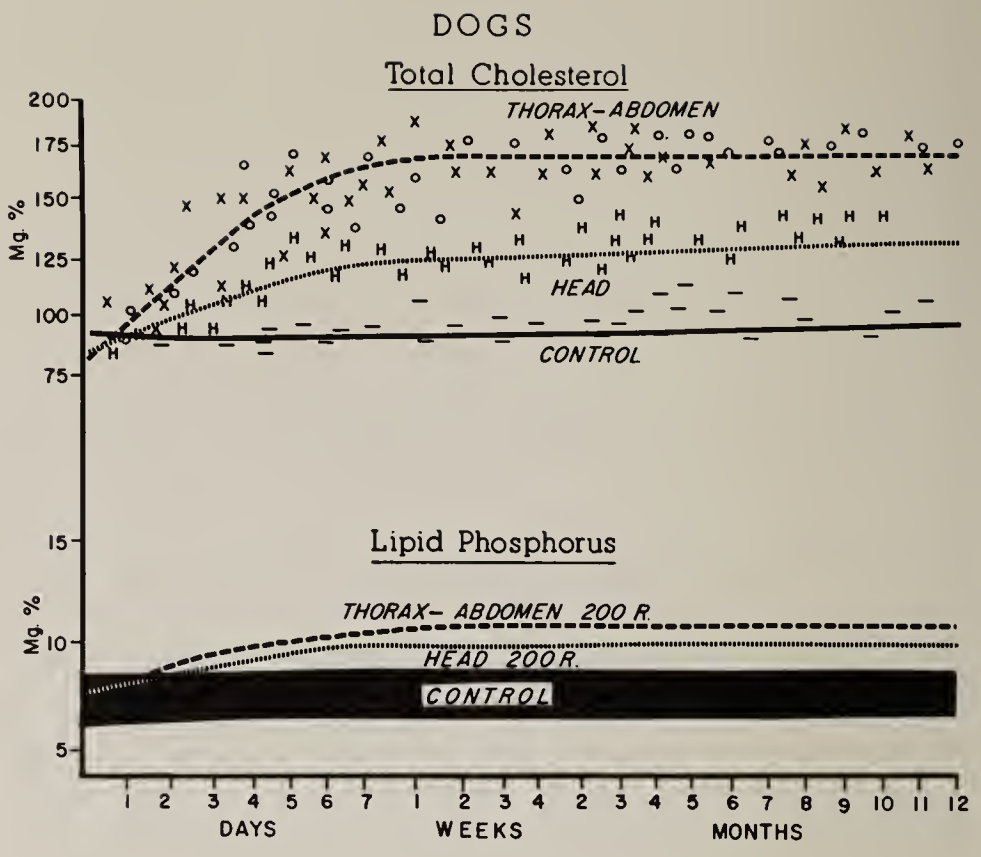
Several dose exposures were selected in an attempt to establish an effective dose range for the hyperlipemia response. Some of these data are expressed in *Figure 1*. The various lines indicate groups of animals according to exposure dose and the changes observed in serum concentrations of lipid. During this time, blood sugar determinations and blood urea nitrogen determinations remained within normal limits. Several determinations of isocitric dehydrogenase, serum glutamic-oxalecetic transaminase, and serum glutamic-pyruvic transaminase were carried out. These results were erratic and did not correlate in any significant way with the changes in serum lipids. The control animals showed no significant rise in serum lipids over a six-month interval. No correlation exists between the pre-irradiation serum lipid levels and the final levels reached. Prediction of radio-sensitivity for the degree of hyperlipemia response in an individual animal is impossible. Significant increases were noted in all serum lipid fractions of animals receiving an "effective dose." This dose is species dependent and this has been confirmed many times. No differences in external appearance were noted in the interval of this study.

The rise in serum lipid fractions involved all the fractions measured. After the initial rise, the hyperlipemia was persistent and in some cases continued to rise along a gentle slope. In the rabbit studies, it made little difference whether the irradiation was delivered as whole-body irradiation or to the thorax or abdomen.

Figure 2 demonstrates that the hyperlipemia was produced in dogs receiving whole-body irradiation, segmental irradiation to the abdomen and thorax, and,



in lesser degree, following irradiation to the head. Again no significant change in blood sugar or blood urea nitrogen was observed during this time, and there is no reliable correlation between the measured enzyme activity and the lipemia. With few exceptions, the cholesterol and neutral fat levels began to rise detectably by 8 hours, and were clearly established by 16 hours. The lipid phosphorus rose within 24 hours. The rises were of greater magnitude in rabbits, but were regularly producible in dogs with doses of 200 r compared to doses of about 400 r in rabbits.



DISCUSSION

These results confirm the previously reported hyperlipemia following whole-body irradiation. They also confirm the hyperlipemia following segmental irradiation. The serum lipid increases are again greatest in the rabbit, but increased levels of various serum lipid fractions have been observed in both species. The difference in the levels of the "effective dose" between these two species might have been anticipated from the known difference in the LD 50 levels for these animals. The approximate 30 day mean lethal dose for dogs is 325 r compared to a 30 day mean lethal dose of 800 r for rabbits[5]. The times at which the lipemia appeared and the curves of the subsequent lipemia are of importance. Previous studies have demonstrated the relatively early appearance but have not been continued for this length of time. Di Luzio and Simon[6] observed survival in their lipemic animals for thirty days. Steadman and Thompson[7] followed their animals for three days. The Atomic Energy Commission group reported on animal follow-ups extending over nine months, but did not follow serum lipids.

Hanel *et al*[8] demonstrated a moderately decreased synthesis of cholesterol in the first six hours following whole-body irradiation. Gould *et al.*[9] reported that the specific activity of rat liver cholesterol is increased six- to tenfold twenty-four hours after 2,400 r whole-body x-irradiation. They correlated the rise in specific activity with the level of irradiation to levels of 300 r. The present results with significantly smaller and segmental types of irradiation indicate that increased liver lipid synthesis may not be the only mechanism operating. Hayes and Hewitt suggested that increased requirements for fat by irradiated animals or damage to a "clearing factor" may operate as well. The fact that segmental irradiation, including irradiation to the head, produced hyperlipemia suggests the possibility of a humoral factor. The elevation of lipid fractions following irradiation to the head requires explanation. The tissue volume of the head is large, but the fact that the lipid elevations persist for a long time indicates continued formation of these compounds, presumably in the same areas where such production is normally accomplished. That there is a sharply rising curve over the first 48 hours, rather than a nearly immediate rise, to a new relatively fixed level suggests an indirect effect on sites of lipid formation.

An early adrenal response to external irradiation has been shown by the de-

pletion of ascorbic acid in the adrenals and an elevation in plasma 17-hydroxy-corticosteroids. Repeated injections of cortisone have also produced hyperlipemia. Traumatic injury sometimes produced elevations in lipoproteins of rabbits. Weiner *et al.*[10] stated that active infection and necrosis may be important factors in producing lipid alterations following irradiation. The trauma and its sequelae are extensive and obvious in their methods of producing hyperlipemia. In the dose ranges employed in this study and in the objective absence of local tissue necrosis, some other factor is postulated.

The dynamic state of adipose tissue has been the subject of much speculation and some evidence. To explain hyperlipemia as "injury response" is to beg the question. Many factors have been evoked to explain the mobility of fat. Peters[11] reviewed the subject in 1951, and Wertheimer and Shapiro[12] had clearly demonstrated the dynamic features of the adipose reservoir. Hormonal influence by the pituitary, adrenal cortex and medulla, pancreas, ovary, and thyroid have been demonstrated. The neuroanatomy of supply to these reservoirs is poorly delineated, but the mobility of the fat depot is in some way dependent upon the integrity of the neural as well as the vascular supply.

Starvation, administration of insulin, tri-iodothyronine, growth hormone, epinephrine and nor-epinephrine have all been demonstrated to have effects in the early mobilization of lipids from depots. We shall continue to study the effect on lipid fractions following irradiation to equal tissue volumes in extremities and in the head.

SUMMARY

The already documented hyperlipemia following whole-body irradiation has been confirmed in rabbits and in dogs. It has been demonstrated that the hyperlipemia tends to remain or to rise slightly over one year. The hyperlipemia is not correlated with blood sugar or with blood urea nitrogen determinations. No correlation can be demonstrated with the erratic changes in isocitric dehydrogenase, serum glutamic-oxalacetic transaminase, and serum glutamic-pyruvic transaminase.

In addition, hyperlipemia has been obtained following segemental irradiation, including localized irradiation to the head. The nature and duration of these types of hyperlipemia may shed light upon some of the factors controlling fat mobility and metabolism.

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COMPLICATIONS OF ABDOMINAL AORTIC GRAFTS AND THEIR MANAGEMENT

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This report is designed to review our experience as a basis for critical evaluation of late complications of abdominal aortic surgery and to explore methods of preventing some of the major complications of grafting procedures.

Since March, 1952, 660 patients were operated on for arteriosclerotic lesions of the aortoiliac system, using homograft or prosthetic material for repair. Of these, 240 patients had aneurysms and 420 had occlusive lesions. Serious complications involving graft or anastomosis occurred in 28 patients after recovery from the immediate postoperative period *(Table I)*. Not included are early bleeding from graft or suture line, thrombosis of graft and complications not related to the graft. Three cases of colon necrosis in the early postoperative period are reported because of the grave nature of this condition.

Incidence of complications is much higher in aneurysms (18 of 240, or 7.5 per cent) than in occlusive lesions (12 of 420, or 2.9 per cent). This difference is probably related primarily to the severity of degenerative changes of the aortic wall in aneurysm. The majority of patients who had intra-abdominal graft-related complications died despite surgical intervention. Only 9 of 21 patients in this category survived. On the other hand, none of a group of 8 with extra-abdominal rupture of the suture line died.

Delayed Rupture Without Bowel Involvement:

Failure of a suture line or rupture of the aorta occurred in seventeen patients. In patients treated for abdominal aneurysm, eight cases of intra-abdominal bleeding occurred, whereas there was only one case of dehiscence of the femoral suture line. Three of the eight patients had originally had emergency surgery for ruptured aneurysm. In most, the quality of the proximal aorta had been found to be extremely poor, and in four removal of calcified plaques was necessary to complete the proximal anastomosis.

Diagnosis of intra-abdominal bleeding was suspected in five patients seen. Of four operated on, the proximal aorta was considered inoperable in two, while repair was successful in the other two. Autopsy findings of suture line disruption in three patients were relayed to us by the family physicians.

Seven of eight patients in whom rupture of a femoral anastomosis occurred had been treated for occlusive lesions. In four, femoral endarterectomy had been necessary before grafting could be accomplished. Staphylococcus albus, coagulase-negative, was cultured in the wounds of four patients. In one with evidence of systemic Staph. albus bactere-

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mia, there was dehiscence of both femoral suture lines. These four patients responded favorably to surgical repair and the suture lines healed despite the contaminant. In two patients thrombosis of the graft followed repair of false aneurysm and above-knee amputation became necessary in each.

Diagnosis—The possibility of intra-abdominal graft failure should be considered when abdominal pain and a pulsatile mass are noted. Aortogram may, in certain instances, be misleading if the false aneurysm is filled with clots. Emergency surgery is indicated, since otherwise the outcome is universally fatal.

The diagnosis of rupture of femoral anastomosis is obvious in the presence of a tender, pulsatile, rapidly enlarging mass at the level of the groin. Despite this revealing sign, erroneous diagnoses and attempts at incision and drainage were made in two patients before readmission to our service.

fistula was repaired, with temporary success of 41 days; a subsequent recurrence was again repaired and a Dacron prosthesis implanted. The communication recurred a third time and the patient died. In another patient there was contamination of the operative site by intestinal content when the duodenum was inadvertently entered during its separation from the aorta. Although a clinical infection did not result, a fatal communication between the proximal suture line and the third portion of the duodenum developed two months after surgery. The third patient with ruptured aneurysm had massive retroperitoneal hemorrhage. Following repair, an abscess developed in the retroperitoneal hematoma, finally resulting in erosion of the suture line and hemorrhage from a fistula between the proximal anastomosis and duodenum.

Aneurysm resection in another patient was followed by good health for three years. Then attacks of abdominal cramps

TABLE I

MAJOR COMPLICATIONS OF 660 ABDOMINAL AORTIC GRAFTS

	Aneurysm (240)			Occlusive Lesions (420)		
	No. Patients	Interval	No. Survived	No. Patients	Interval	No. Survived
Delayed Rupture	9	1-24 mo.	3	8	1-23 mo.	7
Aortointestinal Fistula	6	1-43 mo.	2	0	—	—
Infection of Graft Site	1	15 mo.	1	6	1-24 mo.	3
Necrosis of Colon	2	2-5 days	0	1	4 days	1
	18			15 (12 patients)*		

*Fifteen complications occurred in 12 patients. One individual had disruption of distal suture line, infection of the graft and bowel necrosis. In another in this group disruption of suture line was complicated by secondary infection.

Aortointestinal Fistula:

This complication was noted in six patients. All were originally seen for management of abdominal aneurysm, three of which had ruptured. In one patient, spontaneous rupture had occurred into the third portion of the duodenum, and reconstruction was made with a homograft. This patient presented a history of intermittent gastrointestinal bleeding for three weeks. An aortoduodenal began, with relief accompanied by passage of a tarry stool. Gastrointestinal studies gave essentially negative results, and aortogram showed some irregularity at the level of the proximal stuture line. Laparotomy, including gastrotomy and duodenotomy, did not reveal the site of bleeding. Eight days later another celiotomy revealed the source only after separation of graft from attached jejunum. Repair of the communication between the proximal suture line and the first part of the jejunum was insecure because of

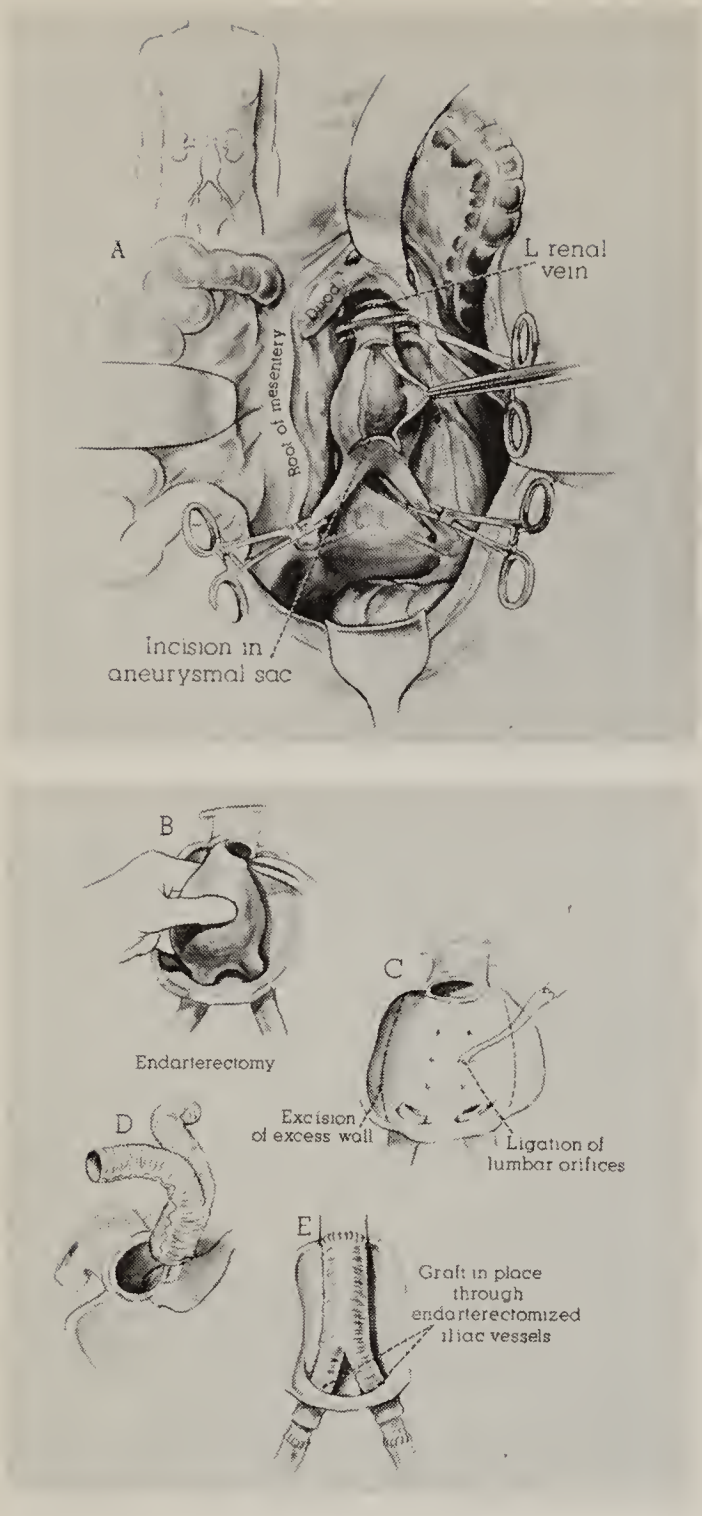


Fig. 1.—Technique of resection of abdominal aortic aneurysm.

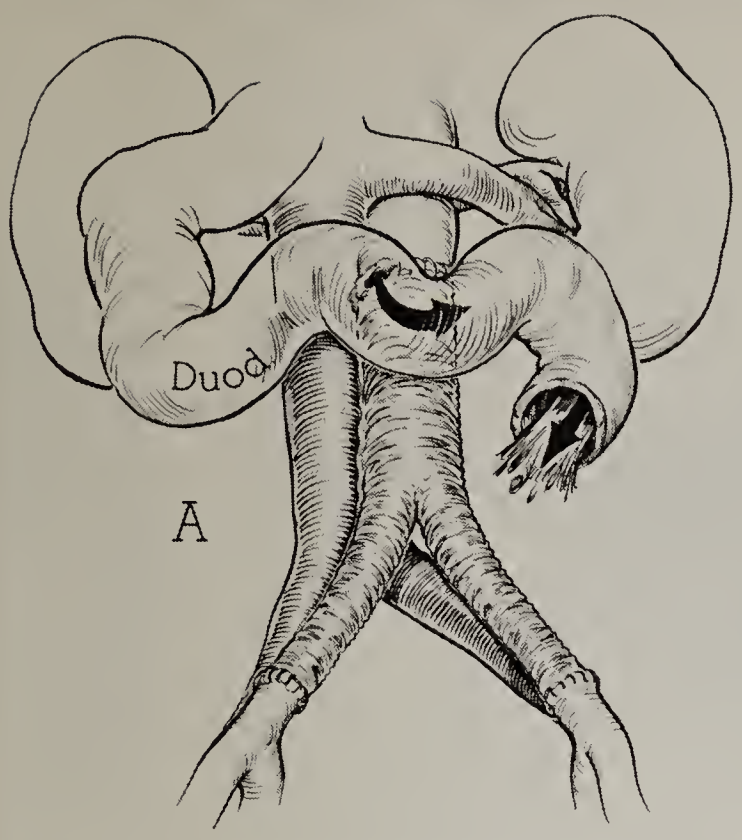
virtual absence of the posterior aortic wall. The patient's condition precluded extending the resection to include the visceral branches. Bleeding recurred a week later with fatal result.

Prevention of Suture Line Dehiscence and Aortointestinal Fistula:

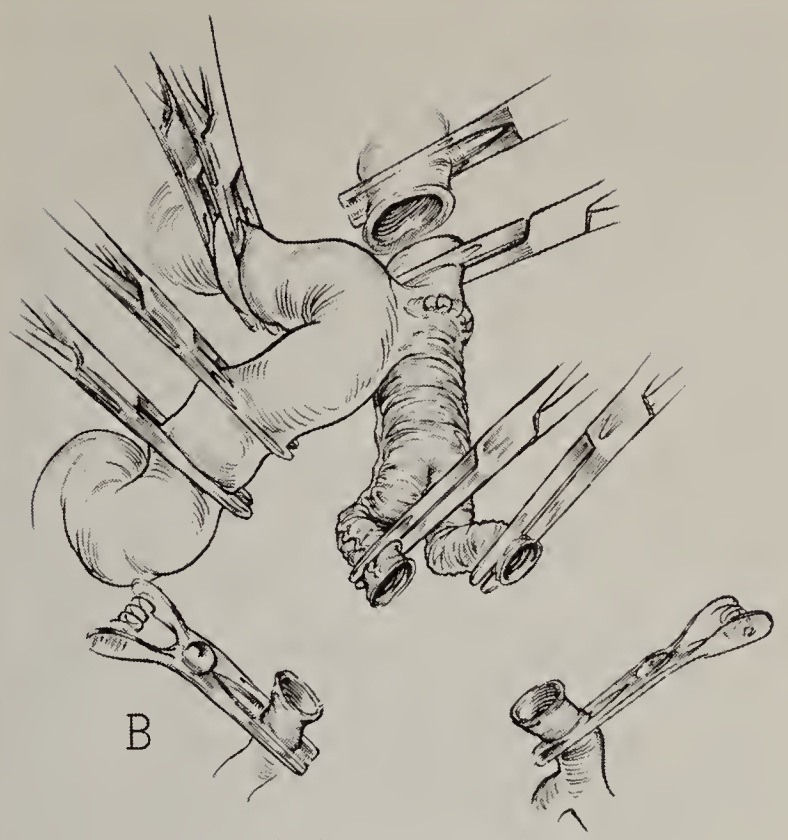
Recent pertinent literature [1,3,4,5,7] and a consideration of our experience lead us to believe that three safeguards are available for prevention of these complications: *(1)* a meticulous and secure proximal suture line; *(2)* avoidance of injury to the gastrointestinal tract and prevention of contamination of the operative site; and *(3)* interposition of viable tissue between the proximal suture line and the duodenum where they lie together in the retroperitoneal space.

In order to fulfill these requirements, we have adopted certain technical maneuvers which appear to be extremely helpful. The technique provides a more secure proximal suture line and aids in separation of the graft from the intestinal tract *(Fig. 1)*. The aneurysm wall is incised longitudinally after proximal and distal control *(A.)* A thorough endarterectomy of the sac is done *(B)*, and lumbar branches are suture-ligated from within the sac *(C)*. The proximal end is prepared by removal of atheromatous plaque, if required. The posterior wall is left attached to prevertebral tissue. A dacron graft of proper size is selected and sutured to the proximal end with deep bites *(D)* of the available tissue. Excess portions of the sac are excised *(E)* and the remaining tissue approximated to cover the suture line and entire graft *(F)*. Limbs of the graft are passed through the endarterectomized common iliac vessels. The posterior peritoneum is then repaired *(G)*, thus separating the intestine from the aorta by two layers.

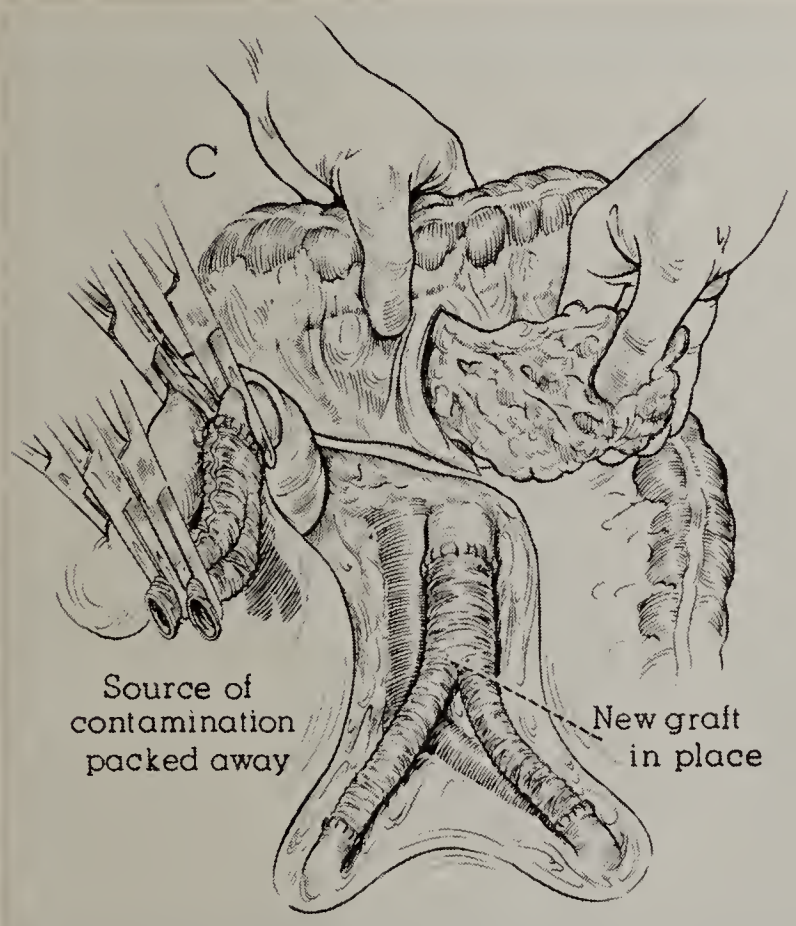
Surgical management of aortointestinal fistula is extremely disappointing, and every effort should be made to prevent this complication. The disrupted suture line is often so close to renal artery branches that proximal control is difficult and contamination of the new graft



Aorto-intestinal fistula



Method of en bloc excision



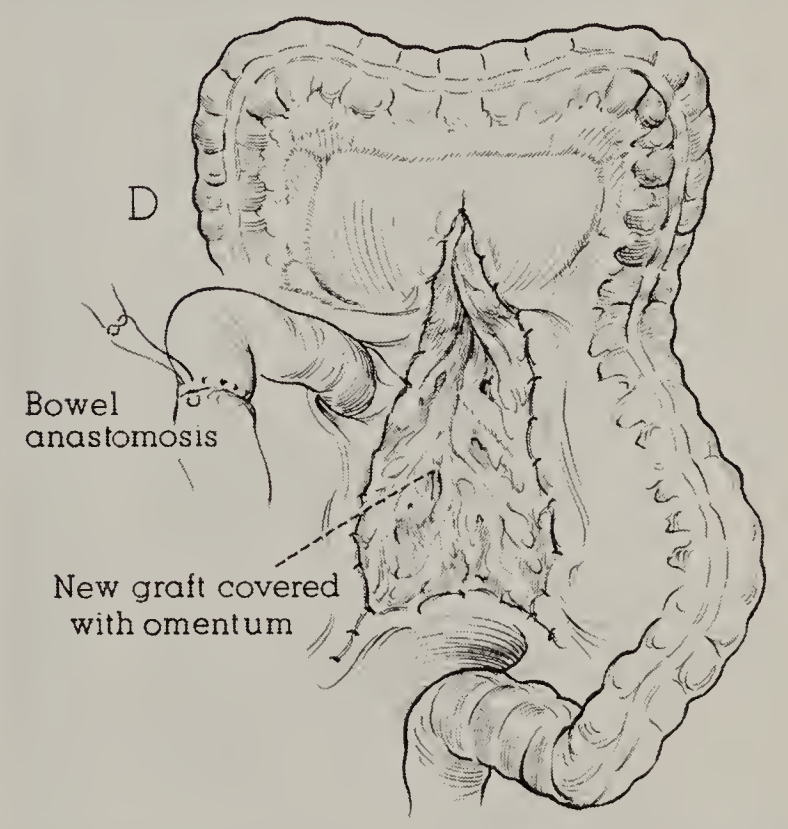


Fig. 2.—En bloc resection of aortointestinal fistula.

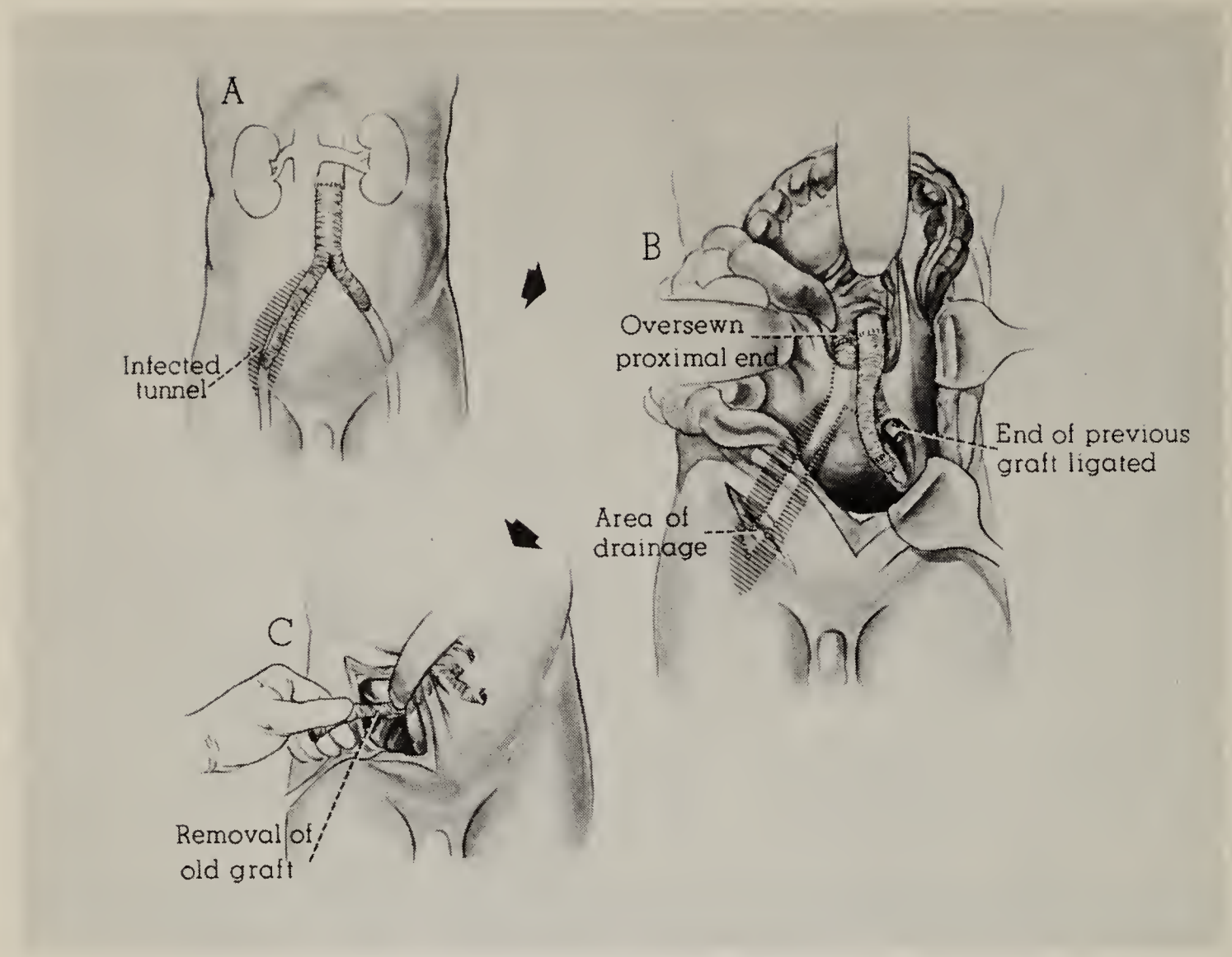


Fig. 3.—Removal of an infected graft.

by intestinal juices is almost automatic. Occasionally, when the fistula is low enough for adequate dissection of the proximal aorta below the renal branches, en bloc excision of the fistula is preferred to avoid contamination of the graft and its anastomosis *(Fig. 2)*. After the aorta is restored with a graft, omentum is brought down to cover it, and only then is the necessary bowel segment removed and the intestine repaired. This technique has been used in one patient who is alive and well twelve months after surgery.

Infection of Graft Site:

Success of grafting in the presence of infection is unlikely, and if a suture line is involved in the infection, its dehiscence seems unavoidable.

One patient had a Staph. aureus, coagulase-positive, infection about the graft in the right groin. The abscess was drained and antibiotics given. Inflammation subsided and the graft remained functional during a thirteen month follow-up. In two other patients infection gradually ascended and involved the entire length of prosthesis. In one, hemorrhage from the aortic suture line caused death, while in the other the graft eroded into the duodenum. Of unusual interest is the latter patient, who had infection of the right limb of a bifurcation aortofemoral by-pass graft and who succumbed to bleeding through the substances of the graft as it lay exposed within an ulcer in the duodenum. Autopsy showed the proximal end of the graft within the lumen of the duodenum and the upper anastomosis intact and outside the intestine. The pseudo-intima had separated from the graft and piled up at the level of the bifurcation. Complete blockage of the right limb of the graft thus resulted. It is assumed that digestive juices caused the separation of the pseudo-intima and massive hemorrhage.

In four patients it was necessary to remove the infected graft in order to control a draining sinus and intermittent bleeding. Operation to by-pass the infected graft *(Fig. 3)* was carried out in three. During implantation of the by-pass, the inflammatory mass was carefully avoided. The infected graft was removed later and, in each patient, the abscess cavity healed. In the fourth patient, who had an iliofemoral by-pass graft, a persistent abscess developed in the left groin ten months after surgery. After removal of the graft the wound healed rapidly. Six months later he had massive lower gastrointestinal bleeding; emergency surgery revealed a fistula between the left external iliac artery and the sigmoid colon. The left external iliac artery was ligated immediately above the communication and bleeding was controlled.

Necrosis of the Colon:

Necrosis of the colon following surgery for aortoiliac disease occurs infrequently and only after some unusual circumstances. Marginal arteries form a complete arcade, and ligation of one or two sources of blood supply may be well tolerated, provided the continuity of the arcade is preserved. Steward and Rankin[6] in a study of colon circulation found the middle colic artery absent in five per cent of their specimens. Practical experience has demonstrated the adequacy in most cases of the collateral circulation between the sigmoidal branches of the inferior mesenteric artery and the middle hemorrhoidal branches of the internal iliac artery.

It is generally accepted that ligation of the inferior mesenteric artery is well tolerated; when ligation is combined with interference of pulsatile flow into the internal iliac arteries, occasional instances of necrosis can occur. In twenty patients who, of necessity, had ligation of the inferior mesenteric and the internal iliac arteries, there was only one instance of colon necrosis.

Colon necrosis developed in three of our series of 660 patients. Diagnosis was made in two patients when bloody drainage from the rectum and localized abdominal tenderness prompted use of the proctoscope. Necrosis of the recto-sigmoid was a coincidental finding at autopsy in the third, who died of myocardial infarction five days postoperatively.

In one patient the basis of necrosis of the left colon was interference with the blood flow through the inferior mesenteric and left iliac system. The condition was further complicated by absence of the middle colic artery. The patient recovered after the involved segment of colon was removed.

The other patient had a ruptured abdominal aortic aneurysm. Both internal iliac arteries were involved and could not be preserved. Postoperatively, hypotension was present and marked abdominal distention required re-exploration. Bleeding from the junction of the limbs of the graft was controlled. A day later localized abdominal tenderness and bloody rectal drainage prompted proctoscopy. Necrosis of mucosa was noted as far proximally as could be visualized. At surgery the same day the sigmoid colon did not appear grossly necrotic. Repeat proctoscopy under anesthesia confirmed previous findings. The colon was removed up to the splenic flexure, and cut section showed necrosis of the entire mucosa and patchy involvement of the muscularis. This patient made a satisfactory recovery from the resection but died twelve days later following aspiration of gastric contents.

We believe there are certain valuable considerations to be noted from a review of this material. We found ligation of the inferior mesenteric and internal iliac arteries is generally tolerated by most patients. A high degree of alertness is necessary to diagnose colon ischemia in the early postoperative period, and proctoscopic examinations should be made in the presence of localized abdominal tenderness, unexplained hypotension, marked abdominal distention, diarrhea, and particularly melena. There may be significant mucosal necrosis without noticeable change in appearance of the bowel serosa.

To preserve viability of the colon, the patency and presence of a middle colic artery must be ascertained before aortic resection. Ligation of the inferior mesenteric artery should be done immediately adjacent to the aorta to avoid interference with the arterial arcade, and every effort should be made to restore pulsatile arterial flow into the internal iliac arteries.

DISCUSSION

It is recognized that many complications of aortic grafts cannot be prevented because of the nature of the disease and degenerative changes of the aorta (1, 2, 5). However, closer attention to technique of aortic grafting, a high degree of alertness in detecting postoperative and particularly late complications and proper management of complications may improve the outlook in aortoiliac surgery. Since disruption of the proximal suture line is a serious and relatively common occurrence after abdominal aneurysm resection, every effort should be made to ensure a secure anastomosis. The technique described serves to separate the graft and suture line from the duodenum by viable tissue and adds to the strength of this anastomosis.

Dissection of the bowel and section of adhesions should be done carefully to avoid contamination of the field by intestinal content. If the duodenum is intimately attached to the wall of the aneurysm, it is better to leave that part of the sac attached to the duodenum than to attempt separation. There should be careful examination of marginal arteries and of the status of the middle colic artery before aortic resection. Evacuation of massive hematoma in the presence of ruptured aneurysm may prevent retroperitoneal abscess formation.

Gastrointestinal bleeding following aortic grafting in a patient with normal gastrointestinal series indicates an aortointestinal fistula. A negative aortogram and abdominal exploration do not exclude the possibility of this complication. To detect the site of fistula, the intestine should be dissected from the graft to the level of communication. En bloc excision of the fistula and maintenance of sterility, when feasible, may prevent recurrence.

The presence of infection around a prosthetic graft is poorly tolerated. To save the patient from the hazards of massive hemorrhage and systemic infection, the graft must be removed.

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THE INCORPORATION OF TRITIATED THYMIDINE INTO THE DNA OF HUMAN BREAST TUMORS IN VITRO

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FRANK R. HENDRICKSON, M.D.

INTRODUCTION

Previous work on the incorporation *in vitro* of formate-C^{14}, serine-3-C^{14}, leucine-1-C^{14}, and methionine-m-C^{14} into normal and neoplastic breast tissue, before and during a course of steroid hormonal therapy, has indicated a close correlation between the uptake of C^{14} and the clinical course of the disease[1, 2]. However, due to the heterogeneity encountered with these tissues and the complexity of interpretation of the biochemical changes due to therapy, it has been felt that a more direct and more specific precursor should be used to correlate tissue metabolism with therapeutic response.

The incorporation of tritiated thymidine into the DNA of tumor cells could give a more definitive answer as to the effects of chemotherapy on intracellular metabolism and mitosis. Thymidine is only incorporated into the DNA of cell nuclei and only during a part of the interphase[3]. Consequently, in any population of cells the specific activity of DNA will depend on the population of cells synthesizing DNA at the time of administration of label. Inhibition or delay of mitosis, even in an exponentially growing population, may delay or inhibit the onset of subsequent cell cycle thus decreasing the proportion of cells synthesizing DNA without necessarily affecting the process of DNA synthesis.

For any chemotherapeutic agent to be effective in the treatment of cancer it should decrease cell multiplication and thus DNA synthesis regardless of its mechanism of action. Therefore, a comparison between thymidine incorporation into tumor nuclei, before and after the initiation of therapy, and the clinical effectiveness of the therapeutic agent should be of value as a means of screening prospective chemotherapeutic agents in humans. Preliminary results are presented here to show the use of thymidine-H^3 incorporation into the DNA of human tumor tissue before and after the initiation of chemotherapy. Comparisons have been made between formate-C^{14} and thymidine-H^3 uptakes into the tumor tissues obtained from the same individuals. Radioautographic techniques are also employed as a means of measuring the percentage of tumor cells undergoing mitosis.

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This work has been supported by N.I.H. Grant CY-3060.

METHODS AND MATERIALS

Normal and neoplastic human breast tissues, obtained from the same individual by surgical biopsy before and after the initiation of therapy, were incubated as tissue slices in a Krebs-Ringer's bicarbonate medium containing 1 μc formate-C^{14} (0.33 $\mu c/\mu M$) or 5 μc thymidine-H^3 (360 $\mu c/\mu M$).

The nucleic acids were extracted from the lipid-free cold TCA-insoluble residue by heating a weighed aliquot of this residue with 2.0 ml. of 5 per cent TCA (trichloracetic acid) for 1 hour at 100°C. The quantities of DNA were determined by their reactions to diphenylamine. Tritium, representing DNA-Thymine, was estimated by liquid scintillation counting. 0.5 ml. aliquot of the hot TCA extract was combined with 0.1 ml. of hyamine (Rohm & Haas), 2 ml. of absolute alcohol, and 15 ml. of scintillation fluid, consisting of: 7.5 gms. of 2,5-diphenyloxazole, 75 mg. of 1,4-bis-2-(5-phenyloxazolyl)-benzene, 120 gms. naphthalene, in one liter of 1:1 dioxane-xylene. This scintillation mixture gives a counting efficiency of 12 per cent for tritium. All samples were counted sufficient for an accuracy of less than 5 per cent counting error. The results for tritium are reported as:

$$\frac{\text{counts/min./milligram DNA}}{\text{counts/min./}\mu\text{m of thymidine-H}^3}$$

in the incubation mixture.

The results which have been obtained have been dependent and limited by the use of surgical biopsy, which have introduced certain mechanical problems which do not lend themselves well to human investigation. Among these are: (1) The amount of tissue necessary for a determination. (2) Inconvenience to the patient for multiple surgical biopsies. (3) Some patients do not have enough tumor tissue available for multiple determinations, especially patients whose tumors have regressed due to therapy. (4) Tissue heterogeneity which make interpretations difficult. In order to overcome some of these problems, techniques have been established which utilize needle biopsies for *in vitro* metabolic studies. A number of needles have been tried and thus far, the Necker* renal biopsy needle has been the most suitable for repeated removal of breast tumor tissue.

Tissues removed by needle biopsy were immediately placed in 3 ml. of Krebs-Ringer's bicarbonate medium containing 5 μc thymidine-H^3 and incubated for one hour at 38° under an atmosphere of 95 per cent O_2, 5 per cent CO_2. The incubations were terminated by placing the tissues into warm formalin for not less than 24 hours. The tissues were washed in cold water overnight in order to remove any remaining free thymidine. The tissues were mounted in paraffin, 5 micron tissue sections cut, mounted on microscope slides, and deparaffinized in xylol. Some sections were stained by means of the Feulgens reaction[4] before making the radioautographs.

In a darkroom the liquid emulsion, NTB_3 (Eastman-Kodak) was brought to a temperature of 43° in a water bath. The tissue sections were placed in the emulsion for approximately 15 seconds and allowed to dry in air before storing in black slide boxes containing dririte tubes. The boxes were sealed with black plastic tape and stored at 4°C until developed. Three weeks exposure to the tritium labeled tissues were usually required to develop sufficient granules over the nuclei. After development and fixation the radioautographs were stained with Harris' Hematoxylin-eosin and mounted for microscopic evaluation. The radioautographs were subjected to cell counting and the results expressed as:

$$\frac{\text{number of tumor nuclei with grains}}{\text{total number of tumor nuclei counted}} \times 100$$

These results were compared with the results obtained by the chemical procedures described above.

RESULTS AND DISCUSSION

The *in vivo* incorporation of thymidine-H^3 into DNA has been studied by Johnson *et al* [5,6] by the use of autoradiography and has proven useful in the study of rates of cell proliforation in a variety of tissues, including human tumors. These authors[7] have also studied the *in vitro* thymidine incorporation into human tissues and have studied 3 cell populations: non-neoplastic duct epithelium, fibroadenoma, and invasive duct cell carci-

*(Collin Gentile & Co., Paris, France)

noma of human breast. Similar rates of proliferation were found in carcinoma cells and duct epithelium in the same individual.

The effects of estradiol therapy on the *in vitro* incorporation of thymidine-H^3 into the DNA of human breast cancer has been studied. Comparison has been made simultaneously with the *in vitro* incorporation of formate-C^{14}. Data are presented in Figure 1 showing the effects of estradiol therapy on the uptake of formate and thymidine into tumor tissues taken from four patients before and one week after the initiation of therapy. Patient *No. 1* had a slow clinical progression of disease before the initiation of therapy and showed no clinical response to estradiol administration. Patient *No. 2* had a rapidly growing tumor prior to the initiation of estradiol therapy. This patient obtained a marked regression of disease from continual estradiol therapy which was of 13 months duration. Patient *No. 3* was classified as moderately progressing with questionable benefit from estradiol therapy. The last patient had a very slow growing tumor which remained static during the period of estrogen administration. In each instance the incorporation of both formate and thymidine was a reflection of the clinical response to therapy. It is of interest that both formate and thymidine respond to estradiol to approximately the same extent, especially when the formate uptake is more a reflection of the rate of protein metabolism, while thymidine uptake reflects DNA synthesis and possibly the extent of mitosis.

The results of long term administration of estradiol are shown in Figure 2

THE EFFECT OF ESTRADIOL ON THE INCORPORATION OF FORMATE—C^{14} AND THYMIDINE—H^3 INTO HUMAN BREAST CANCER

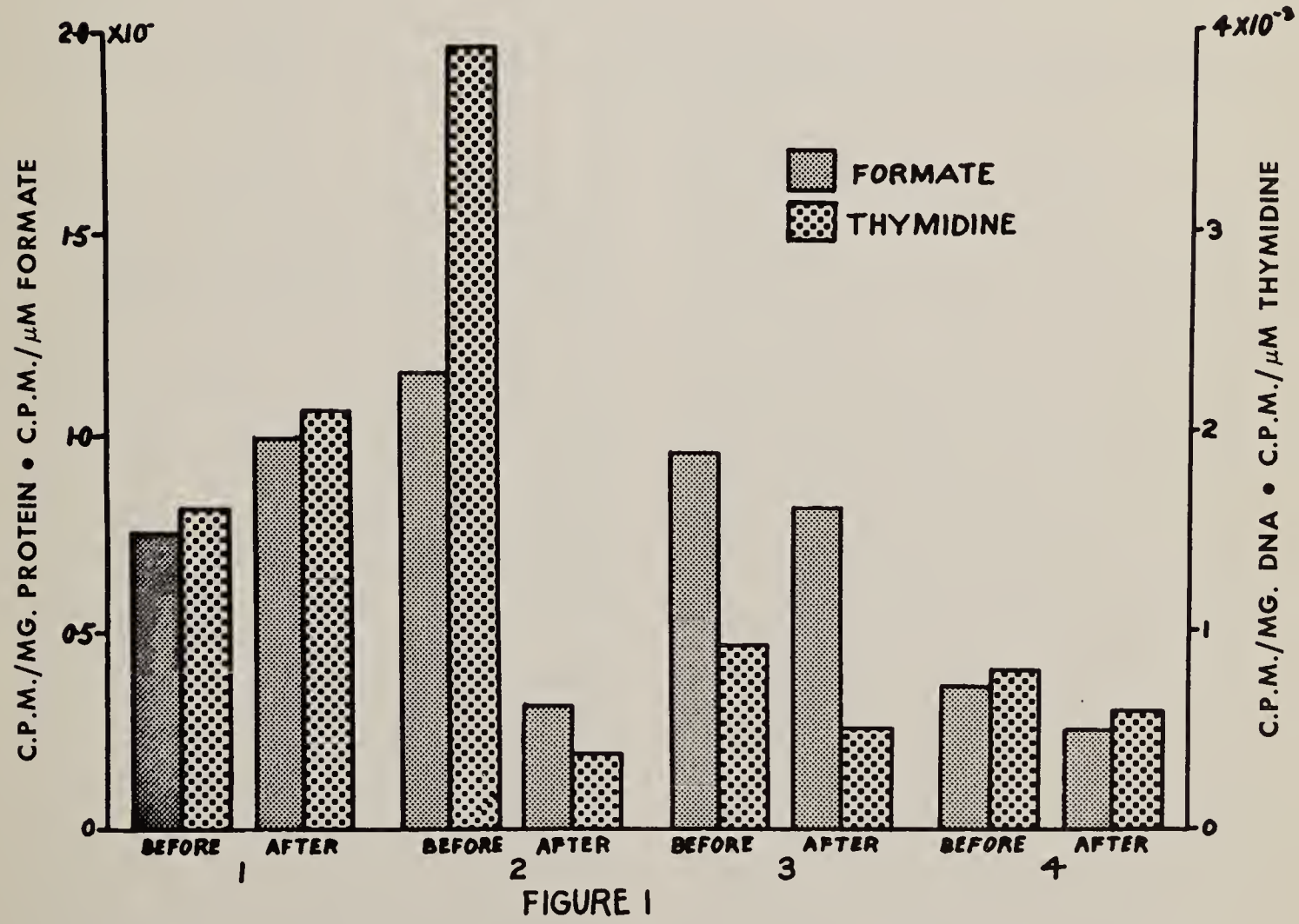
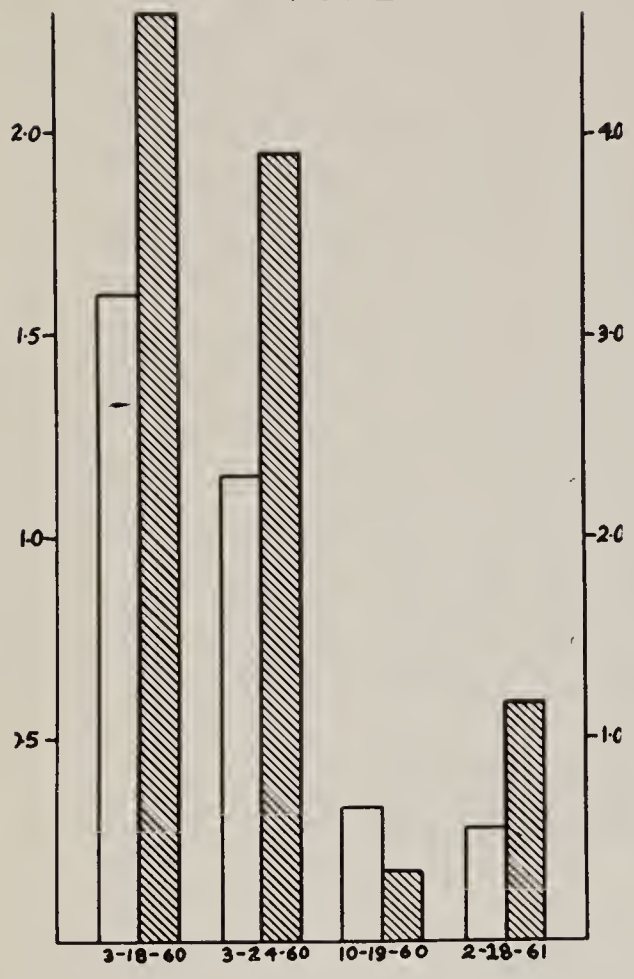


FIGURE 1

INCORPORATION OF FORMATE—C^{14} AND THYMIDINE—H^3 INTO HUMAN BREAST CANCER.

FIGURE 2



where a patient with a very rapidly growing tumor was placed on estradiol and obtained a regression of eleven months duration. However, four months later this patient showed clinical symptoms of progression which seems to have been more accurately reflected by thymidine incorporation into DNA.

The combining of histochemical and radiographic techniques with those usually employed in biochemical investigation should greatly enhance the ability to more accurately interpret these results. A tendency has arisen in biological research to measure the specific radioactivity of components and to equate such measurements with synthesis, turnover, or even growth rate. Certain factors, however, are frequently neglected. For example, if labeled thymidine is added to a population of living cells, the specific radioactivity of the DNA after a certain time will depend on the proportion or fraction of the whole population of cells synthesizing, such as: 100 per cent of cells synthesizing at half the normal rate, or 50 per cent of cells synthesizing at normal rate, or 25 per cent of cells synthesizing at twice normal rate will each yield the same specific activities for the DNA isolated.

It has been shown that DNA synthesis only occurs during part of interphase, consequently in any population of cells the specific activity of DNA will depend on the population of cells synthesizing DNA at the time of administration of label. Therefore, the timing of incubations becomes an important factor in evaluating results of specific activity determinations.

TABLE I

THE EFFECT OF X-IRRADIATION ON THE INCORPORATION OF TRITIATED-THYMIDINE INTO TUMOR DNA**

TIME DAYS AFTER X-RAY*	C.P.M. / MG. DNA	PER CENT NUCLEI LABELED
0	11270	16.0
1	12520	19.0
2	4470	8.5
3	3070	8.0
4	1230	4.0
5	710	2.7

*RADIATION DOSE 300r T.D.
**RADIO-SENSITIVE HODGKIN'S DISEASE

In an attempt to study the correlation between the changes in the specific activity of DNA and the per cent of cell nuclei synthesizing DNA, a patient with Hodgkin's disease, having multiple subcutaneous lesions, was subjected to irradiation therapy. Each lesion was subjected to 300 r T.D., consecutively at 24 hour intervals for 5 days. On the sixth day all radiated lesions plus one non-irradiated control were biopsied and incubated with thymidine-H^3. The specific activities of DNA and the per cent of cell nuclei taking up radioactivity were compared. The results are presented in Table I. In spite of the fact that cell

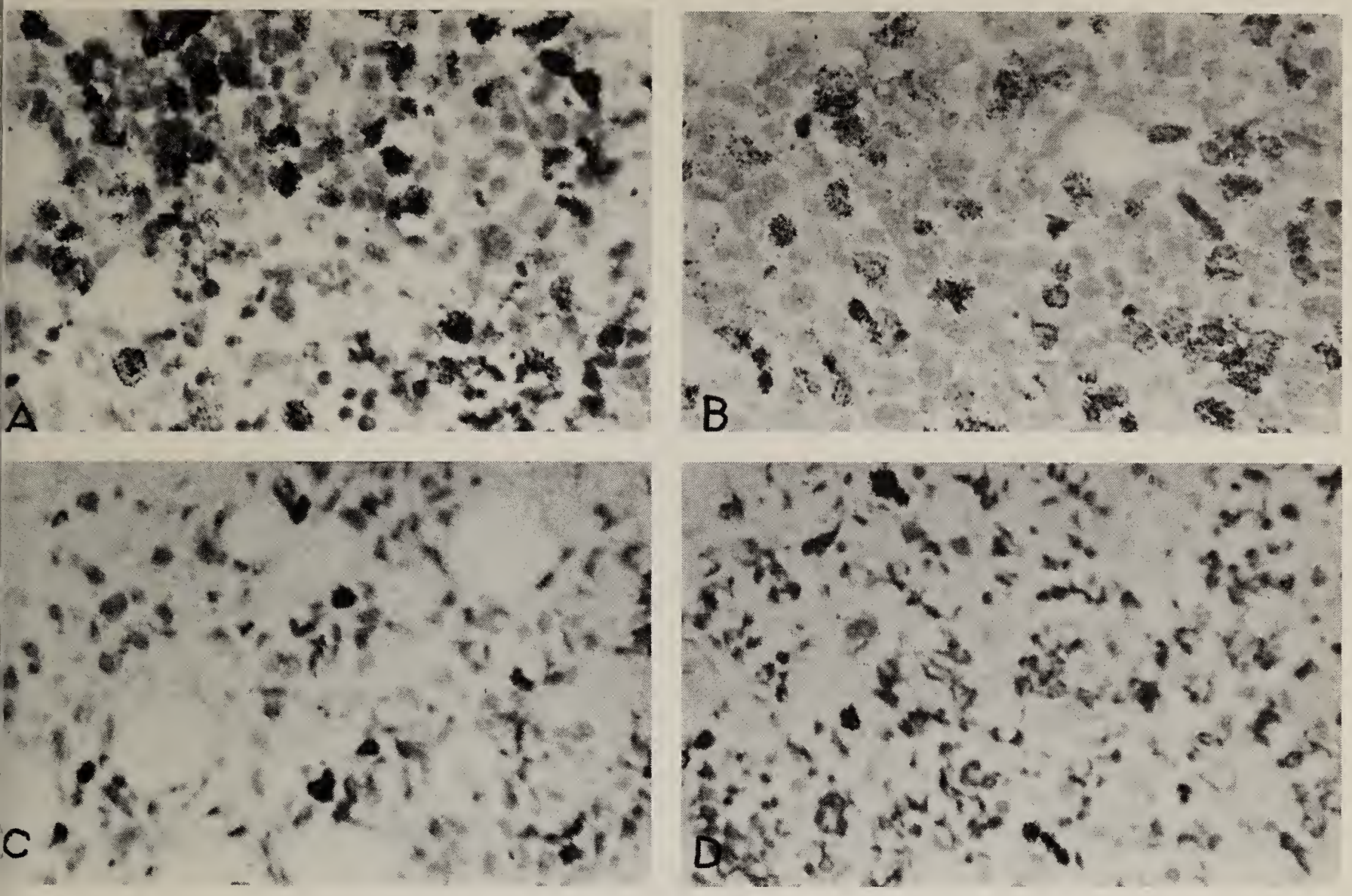


Fig. 3—Radioautographs of tumor tissue showing effects of X-ray on the incorporation of thymidine—H^3 into cell nuclei. A. control, B. 24 hours after X-ray, C. 72 hours after X-ray, D. 120 hours after X-ray.

counting gives only a crude estimate of per cent, there appears to be a general correlation between the specific activity of the DNA extraction from the tissues and the number of cells incorporating thymidine into DNA. It would appear that the decreased specific activity of DNA, following irradiation, is not necessarily due to a change in the rate of synthesis of DNA but to a decreased number of cells entering the pre-mitotic period or the DNA-synthesis phase[7]. This may be due to an increased death rate of the cell population or to a delay in the onset of mitosis. Nygaard and Potter[8] have studied the effects of x-irradiation on DNA synthesis in various tissues of the rat. Their data indicate that the decreased uptake of thymidine caused by x-ray, was in part, caused by a delayed of DNA synthesis. Dewey and Humphrey[9] have reported on the relative radiosensitivity of different phases in the life cycle of mouse fibroblasts. With thymidine-H^3 and the aid of radioautographs, the authors have shown with labeled anaphases, which were synthesizing DNA at the time of irradiation (S period), there was manifested twice as much chromosomal damage as the unlabeled anaphases, which were in the G_1 period at the time of irradiation. Mitotic delay of cells irradiated in different phases of the life cycle were most marked in the G_2 and S periods. Cells in the S period at time of irradiation not only suffered more chromosomal damage but were also discriminated against in subsequent divisions. Thus far there does not seem to be any conflict with the

interpretations of data presented in this report and with other published information.

Radioautographs are shown in Figure 3 from which data were obtained for use in Table I. The four photomicroradioautographs show the production of granules over the nuclei of cells due to the incorporaton of thymidine-H^3 into DNA. The first picture (a) represents the control, (b) 24 hours after x-irradiation, (c) 72 hours after x-irradiation, and (d) 120 hours after x-irradiation.

From the preliminary data presented here, the needle biospy technique for obtaining tissue samples to study the effects of chemotherapeutic agents on the synthesis of DNA, appears practical. It is hoped that the above technique will be useful for the pre-clinical screening of potential chemo-therapeutic agents in human neoplasm.

ACKNOWLEDGMENTS

We wish to express our appreciation to Dr. Charles P. Perlia, Section of Medical Oncology, for the selection and clinical evaluation of patients used in this report and to Dr. Steven G. Economou, Department of Surgery, for obtaining the biopsy specimens used in these experiments. We are indebted to Mr. Arthur Conti, Section of Hematology, for his assistance with the radioautographs used in this work.

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VITREOUS HEMORRHAGE—A RETINAL TEAR UNTIL PROVEN OTHERWISE

Howard L. Wilder, M.D.

There are numerous situations in medicine, where in spite of our best efforts we are unable to produce a successful result. There are also numerous situations where in spite of the therapy given, the patient gets well. And lastly, there are situations where the proper and early diagnosis makes the difference between success and failure and also between a simple versus a more complex type of therapy. This last category fits perfectly the condition of retinal tears without retinal detachment.

If we eliminate the minority causes of retinal detachment which we will call secondary detachments (i.e. those due to intraocular tumor or inflammatory ocular conditions—uveitis etc.), and if we consider only the vast majority of primary retinal detachments where the pathology is due to retinal changes, then it is safe to say that practically all of these detachments are preceded by a tear, hole or break in the continuity of the retina. The interval between the formation of the tear and the retinal detachment is extremely variable and it is true that in some eyes, tears or holes in the retina never do lead to a retinal detachment. Okun showed that approximately 5% of normal eyes at autopsy exhibited retinal hole formation.

It is obvious that the early diagnosis of the retinal tear prior to the formation of the retinal detachment is extremely desirable. The patient can be spared a surgical procedure by having the tear sealed by photocoagulation. This is a process whereby light is converted to heat by the choroid and pigment epithelium of the retina which produces a controlled reaction at the tear site which results in a choreoretinal scar. This seals the retinal tear, thereby preventing the formation of a retinal detachment. The light is directed and controlled by observation thru a viewing ophthalmoscope during the coagulation process. Why is this such a desirable process? First because the retina has not yet detached and, therefore, the cure rate by photocoagulation is in excess of 90%. Second, it is a non-surgical procedure which frequently is done on an out-patient basis, thereby eliminating the hardship of absence from work. The patient is usually absent from his job for only 1-3 days.

Our main concern in this discussion is how can we find these retinal tears in this desirable period—before the detachment occurs? If the patient has no symptoms, then he does not seek help. Unfortunately, many retinal tears form without symptoms. The formation of most retinal tears is in the region of the equator and therefore no visual loss occurs. However, there is a fair number of cases where the formation of the retinal tear does produce symptoms and these symptoms are mainly floaters due to a broken retinal blood vessel and the spray of blood into the vitreous body. If the retinal tear happens to form at a retinal blood vessel it is very likely that the vessel will break and blood will spill into the vitreous. This may be a minute amount of hemorrhage producing floaters only, and com-

From the Department of Opthalmology

These Fungus Photos Show the More Typical Types of Retinal Tears Seen in the Region of the Equator.

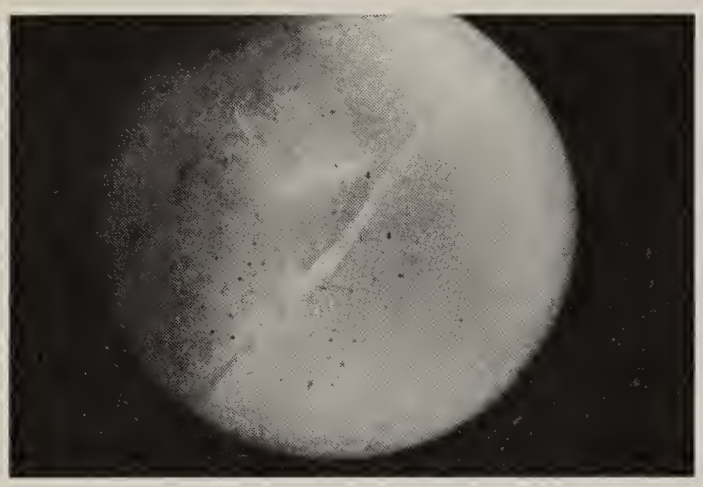

Fig. 1.—This demonstrates a jagged tear in a zone of lattice degeneration. Note the formation of the tear at the fork of the retinal vessels.

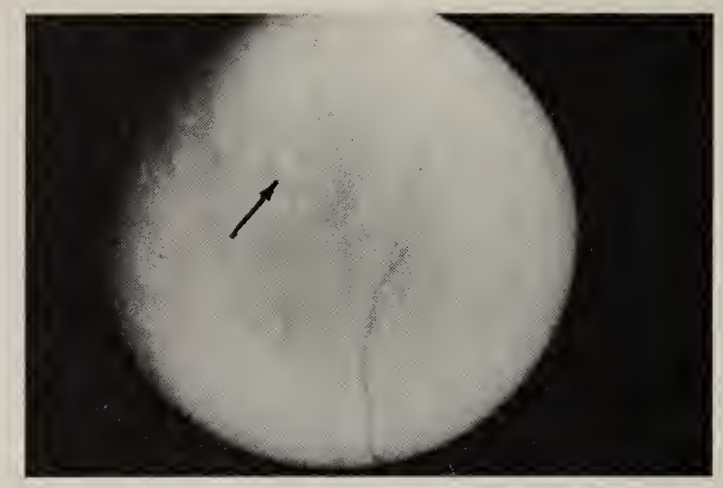

Fig. 2.—This shows a small horseshoe shaped tear.

Fig. 3.—This is the tear in Figure 2 following photocoagulation. Note how much more readily visible the tear becomes after its edges are contrasted by coagulation.

Fig. 4.—This is a large horseshoe or fish-mouth tear just after photocoagulation.

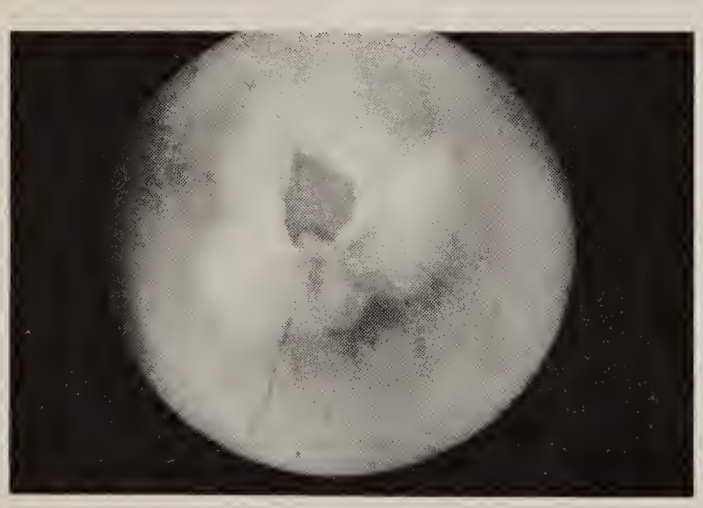

Fig. 5.—This shows a jagged irregular tear in the vicinity of a retinal vessel with some retinal hemorrhage at the tear edge. This was photographed after photocoagulation of the tear edges.

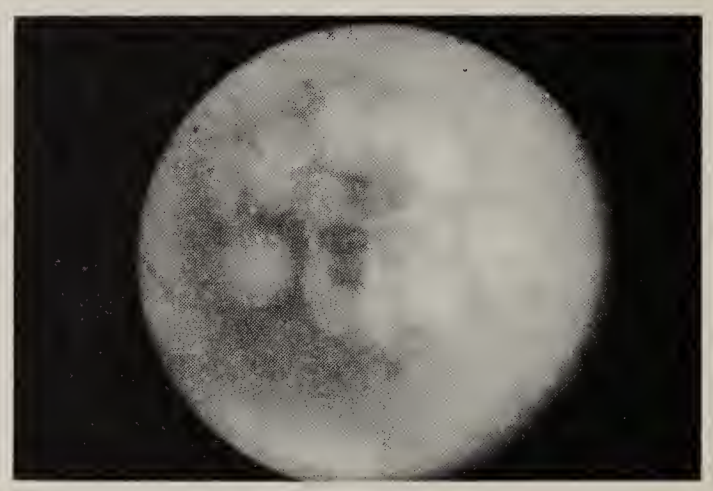

Fig. 6.—This is the appearance of bleeding retinal vessels without tear formation after they have been photocoagulated.

patible with 20/20 vision, or it may be a massive filling of the vitreous with blood and vision reduced to light perception, or it may be any degree between these two extremes. The ideal situation is, obviously, enough hemorrhage to warn the patient something has happened yet not enough hemorrhage to obscure the examiner's view. The least desirable situation is, of course, so much blood that retinal examination is impossible. Fortunately, the use of a binocular indirect ophthalmoscope permits many fundus examinations in such cases whereas with a direct ophthalmoscope no details at all can be seen. This is due mainly to the better illumination of the indirect ophthalmoscope along with its binocular stereoscopic viewing device.

Let us consider for a moment the sequence of events that take place in the patient who has floaters with some degree of visual decrease due to clouding of the vitreous by hemorrhage. If that patient consults his internist, the correct management would be a complete history and physical examination and any out-patient laboratory tests the internist feels are necessary. This is to be coupled with an ophthalmological consultation for fundus study. The incorrect therapy would be admission to the hospital for a large battery of laboratory tests. The reason for this is the key statement of this whole discussion—a vitreous hemorrhage is a retinal tear until proven otherwise. It is my impression that the majority of cases of vitreous hemorrhage are due to retinal tears and from the standpoint of frequency, importance and early therapeutic benefit, this major cause of hemorrhage should be excluded before subjecting the patient to a large series of laboratory tests.

If the patient with this problem now consults the ophthalmologist, the correct management would be an intensive search of the fundus by indirect ophthalmoscopy (and direct ophthalmoscopy if desired) under maximum pupillary dilation. The most important thing to be looked for is a retinal tear. If the vitreous is so clouded that retinal examination is not possible, then the correct thing is to see the patient again—soon. Repeated, frequent examinations are mandatory so that the retina may be seen at its earliest clearing point—prior to the development of a detached retina. The incorrect advice is to tell the patient that the eye is too clouded with blood and that no view will be possible for some time, but that he should return in several months for a second examination. The very common story patients tell us when given this advice is that their doctor was right, the vision did clear and returned to normal (blood had disappeared from the vitreous) and then several weeks later they awakened with a curtain to one side (the field defect of the sudden retinal detachment). If this patient had been examined at frequent intervals, the retinal tear would have been found when the vitreous cleared and prior to the time of the development of the retinal detachment. Treatment by photocoagulation would most likely have cured the situation.

SUMMARY

In cases of vitreous hemorrhage, a plea is made to the internist to consider retinal tears above arteriosclerosis, hypertension, blood dyscrasias and diabetes. A plea is made to the ophthalmologist to consider retinal tear above disciform degeneration, intraocular tumors, trauma, uveitis and new formed retinal vessels. The working rule should be a vitreous hemorrhage is a retinal tear until proven otherwise.

STUDIES OF ANEMIA IN RHEUMATOID ARTHRITIS AND ALLIED CONDITIONS

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Moderate anemia frequently occurs in rheumatoid arthritis, though it is rarely a serious complication. In Nilsson's extensive review[1], 63% of the women with active rheumatoid arthritis had hemoglobin levels of less than 12 grams and 23% had less than 10 grams. In men with the disease, Nilsson reported that 42% had less than 13 grams of hemoglobin and 12% had less than 10 grams. The pathogenesis of the anemia is not clearly understood and some of the offered explanations follow.

In 1955, Dixon and his associates[2] reported that the red cell mass was 5% lower than normal in patients with arthritis and the plasma volume was 22% above normal. This combination produced a marked hemodilution and a falsely low estimate of the red cell mass. Weinstein[3] tended to confirm these findings.

Iron deficiency and possible derangement of iron metabolism have frequently been implicated[4-8]. In rheumatoid arthritis the red cells are normal in size and moderately hypochromic. The reticulocyte count is normal. There is definite hypoferremia with normal to slightly decreased total iron binding capacity. This contrasts with the findings in iron deficiency in which the hypoferremia is associated with an *elevated* iron binding capacity and also differs from chronic inflammation in which the hypoferremia is associated with a marked *decrease* in the iron binding capacity. McCrea reports the presence of stainable iron in the bone marrow of the majority of patients with rheumatoid arthritis[4]. Weinstein[3] confirms this and further describes increased stainable iron in the marrow of many patients.

Patients with rheumatoid arthritis incorporate normal amounts of Fe^{59} into erythrocytes.

Stool blood loss has been measured[9] after tagging circulating red blood cells with Cr^{51}. The chromium tag which is eluted from the red cells appears only in the urine, and any chromium in the stool must come from gastrointestinal bleeding or oozing. In this study of 50 patients with rheumatoid arthritis the estimated daily blood loss in 44 patients was 1 to 4 ml; in 4 it varied from 5 to 9 ml and in 1 patient there was 20 ml. blood loss per day. Normal persons showed no such

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From the Section of Hematology and the Department of Medicine. The research was aided by a grant from the Illinois Chapter, Arthritis and Rheumatism Foundation.

fecal blood loss. This small loss was insufficient to cause any appreciable shortening of the red blood cell survival time, however if continued it could account for a significant loss of iron. As 1 ml of blood contains approximately 0.5 mg. of iron, the above loss can effect an iron loss ranging from 0.5 to 10 mg. per day. Another study has shown that oral salicylates produce regular loss of small amounts of blood in the stool[10]. Until the problem is restudied in patients with rheumatoid arthritis who are not receiving salicylates, it must be assumed likely that the loss may be related to the salicylates rather than to the arthritis. An estimate of the small amounts of blood lost in this way obviously requires methods such as those of Lewis[9]. The usual qualitative guaiac or benzidine tests, even if done daily, can only furnish crude information.

Several studies report the effects of oral or parenteral iron in the treatment of the anemia of rheumatoid arthritis[5,7,11]. Oral iron in general has shown no effect. Richmond, *et al.*[5] treated 26 patients with 5 grams of iron in the form of saccharated iron oxide intravenously and compared these results to those in 20 untreated patients with rheumatoid arthritis. In the treated group the hemoglobin level rose as the functional capacity of the patient increased, as the disease activity diminished and as the erythrocyte sedimentation rate fell. The rise in hemoglobin was not related to the initial degree of anemia, the iron content of the marrow, the mean cell hemoglobin concentration or plasma iron level. The rise was due to an increase in red blood cell count and not to any change or improvement in the hypochromia. It should be pointed out that the total amount of iron given to these patients was greater than the total amount of iron in the body of a large healthy adult man.

In patients with rheumatoid arthritis and anemia, it seems clear that: *(1)* The loss of small amounts of fecal blood is common in patients receiving salicylate therapy but does not significantly influence erythrocyte survival. *(2)* In the absence of marked blood loss, the anemia is not iron deficient and does not respond to oral iron. *(3)* Response to intravenous saccharated iron oxide coincides with improvement in the state of the disease.

Alexander and his co-workers[12] and Freireich, *et al.*[8] have demonstrated that red cells from normal donors show a significantly shortened survival time when transfused into patients with rheumatoid arthritis However, red cells from a patient with rheumatoid arthritis showed normal survival when injected into a normal recipient and essentially normal survival when injected into another patient with rheumatoid arthritis[12]. In these studies the usual signs of overt hemolysis were absent. The reticulocyte count, serum bilirubin and fecal urobilinogen were normal.

In 1959, Weinstein[3] reported Cr^{51} studies of red cell survival in 18 patients with rheumatoid arthritis. In five patients this survival was slightly shortened and in the other 13 patients it was normal.

In summary, there is good evidence that *(1)* autogenous red cells survive normally in patients with rheumatoid arthritis and *(2)* normal, non-rheumatoid red cells have a significantly shortened life span in patients with rheumatoid arthritis.

MATERIAL

The case material comprises 22 patients, of whom four were men and 18 women.* Nineteen had rheumatoid arthritis classed as "Definite" by American Rheumatism Association criteria[13]; two had well developed ankylosing spondylitis; and one, systemic lupus erythematosus. Although hematologic findings in the latter three did not differ significantly from those in the other 19, their results are omitted from tables.

Of those with definite rheumatoid disease, there were two men and 17 women. Duration was from 6 months to 25 years with 13 patients having had their disease between 1 and 7 years.

*We are indebted to our colleagues for permission to study these patients. Dr. E. F. Traut was particularly helpful in this regard.

Elevated sedimentation rates and clinical observation indicated active rheumatoid disease in all cases. Closer assessment of severity was difficult since it was not possible to control many factors extraneous to disease activity *per se*. In addition to their basic rheumatic disease, two patients had had bacterial endocarditis with recovery; two were obese. There was one instance each of psoriasis, acquired hemolytic anemia, peptic ulcer (a surgical fatality), pulmonary tuberculosis, diabetes mellitus, repeated pulmonary infection, urinary infection, and suspected hyperthyroidism.

Both spondylitics were men, with fused spines and no peripheral arthritis. The patient with systemic lupus erythematosus was a woman ill for 6 years whose disease for 5 years had been progressive polyarthritis.

Serological tests for rheumatoid factor were done on 16 patients by latex fixation[14] and on the same number, but not all the same individuals, by the sensitized sheep cell agglutination reaction[15]. By the latex fixation method, three sera were negative (one spondylitic, a male rheumatoid with psoriasis, and a female rheumatoid). Two were "weak" (1:32) and 11 were strongly positive, two each having titers of 1:64, 1:128 and 1:256. Therapy at the time of estimation of erythrocyte survival included the use of corticosteroid drugs, usually prednisone, in 12 of the 19 rheumatoid arthritics; seven were not taking steroids during the period of observation. Of those receiving such medication, six took 10 mg. of prednisone or its equivalent daily; five took 11 to 20 mg, and one took 50 mg. daily. Most patients received aspirin in doses of two to four grams daily.

METHODS

Hemoglobin was measured as cyanmethemoglobin in a 1:300 dilution in a Coleman Junior spectrophotometer at a wavelength setting of 540 mu.

Red blood cell counts were performed on an electronic counter (Coulter, Model A). With this technique, approximately 50,000 cells are counted in a count of 5,000,000 cells cu. mm. reducing the counting error to approximately 0.5%*.

Hematocrits were determined either: *(1)* by the standard Wintrobe technique using balanced oxalate and centrifuging at an RCF greater than 2,000 or *(2)* by a microhematocrit method using heparinized capillary tubes spun in an IEC centrifuge, Model MB, at 11,000 RPM.

Reticulocyte counts were performed using the new methylene blue stain[16].

Serum iron was determined from a tris-iron complex of bathophenanthro line as described by Peters, *et al*[17]. Serum iron binding capacity was determined using an ion exchange resin[18], Amberlite IRA 410. Red cell mass was determined by tagging the patients' red blood cells with $*Cr^{51}$ sodium chromate in a special ACD solution (Abbott's list No 6761). Following room temperature incubation with the sodium radiochromate, a measured quantity of red blood cells was injected into the subject. Ten minutes later a sample of blood was withdrawn and used to determine red cell mass and total blood volume by the dilution principle. This sample was also used as a reference point for red blood cell survival data. Blood samples were then taken at appropriate intervals to determine the rate of destruction. In normal persons, 50% of the Cr^{51} activity is lost in 27 ± 5 days. At the same times that blood samples were drawn, patients were scanned with a scintillation probe over the precordium, spleen, liver and bone marrow (sacrum) in order to estimate areas of accumulation of Cr^{51}. Counts were recorded as organ: precordium ratios. (e.g., if the counting rate over the spleen were 350 cpm and over the precordium were 175 cpm, the spleen: precordium ratio would equal 2:1.) Normally, the organ: precordium ratio is approximately 1:1.

RESULTS

A summary of the hematologic data is presented in *Table I.* It will be noted that none of the patients was markedly anemic. The mean hemoglobin level in the

*Coefficient of variation.

TABLE I

SUMMARY OF HEMATOLOGIC DATA
IN PATIENTS WITH RHEUMATOID ARTHRITIS

		HGB gm/100ml	RBC x10⁶	HTC %	MCV cu. microns	MCHC %	Blood Volume ml/kg	Red Cell Mass ml/kg	Plasma Volume ml/kg	Serum Iron μgm/100ml	RBC $Cr^{51}T$ 1/2 in days
WOMEN (17)	Mean	10.8	4.17	36	89.6	29.9	65.4	21.69	44.33	61.4	25.6
	Standard Deviation	1.5	0.63	5.0	9.7	2	13.21	5.96	8.63	23.8	5.56
	±2 Standard Deviations	7.8-13.8	2.91-5.43	26-46	70.2-109.0	25.9-33.9	38.98-91.82	9.77-33.61	27.07-61.59	13.8-109	14.48-36.72
	Range	8.2-14.0	2.9-4.97	26-45	72-106	27-34	41.4-95.8	13.1-36.2	26.2 59.6	25-112	6-31
MEN (2)	Mean	8.5	4.03	29.5	79	30	100.6	29.7	71.0	33	25
	Range	8.1-8.9	3.55-4.5	28 31	—	—	95.6-105.7	28.7-30.6	66.9-75.1	23-43	23-27

women was 10.8 gm and the hematocrit 36% as compared to normal values of 14 ± 2 gm and 42 ± 5%, respectively[19].

In women, the mean red cell mass, measured by the Cr^{51} tag, was 21.7 ml./kg. as compared to the normal mean in our laboratory of 23 ml/kg. The mean plasma volume of 44.3 ml/kg is only slightly greater than the normal of 43 ml./kg.

In the two men studied, the mean hemoglobin of 9.4 gm and the hematocrit of 32% are clearly low. Note, however, that the red cell mass is 28.9 ml./kg. as compared to the normal of 30. The plasma volume of 66 ml./kg. is appreciably greater than the normal of 46 ml./kg. This implies that the "anemia" is spurious, the result of hydremia.

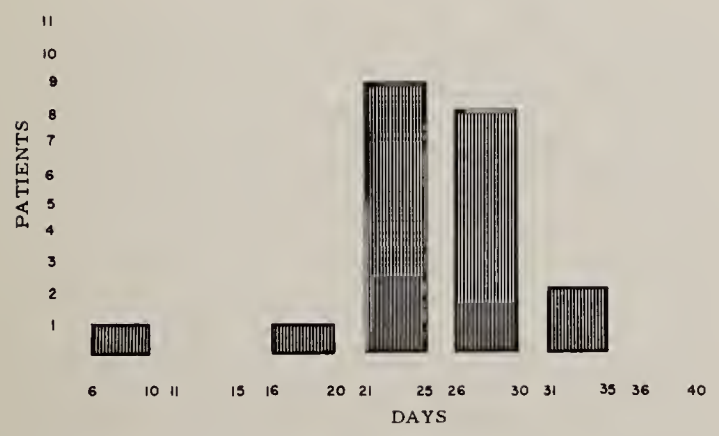

Fig. 1—CR^{51} T-½ red blood cell survival in 19 patients with rheumatoid arthritis.

Our findings agree with the statement that the red cells are normal in size and are slightly hypochromic and there is a mild hypoferremia.

The red cell survival time was normal in most patients. *Figure 1* illustrates the frequency with which different Cr^{51} half times were found. In *Figure 2* the red cell survival is compared to several other measurements. It is easily noted that the erythrocyte life span is unrelated to the presence or absence of rheumatoid factor *(Fig. 2A and 2B)*, the administration of corticosteroids *(Fig. 2C)* or the level of hemoglobin *(Fig. 2D)*.

There was one patient with a red cell survival time of 6 days. All studies indicated that this was the manifestation of an idiopathic acquired hemolytic amenia unrelated to rheumatoid arthritis.

COMMENT

We found no evidence that the anemia of rheumatoid arthritis is severe or of hemolytic origin. In only one subject, the patient with acquired hemolytic anemia referred to in the previous paragraph, does it seem reasonable to explain any anemia on a shortened red cell survival and in this case, the hemoglobin was 9 gm. with a hematocrit of 30%.

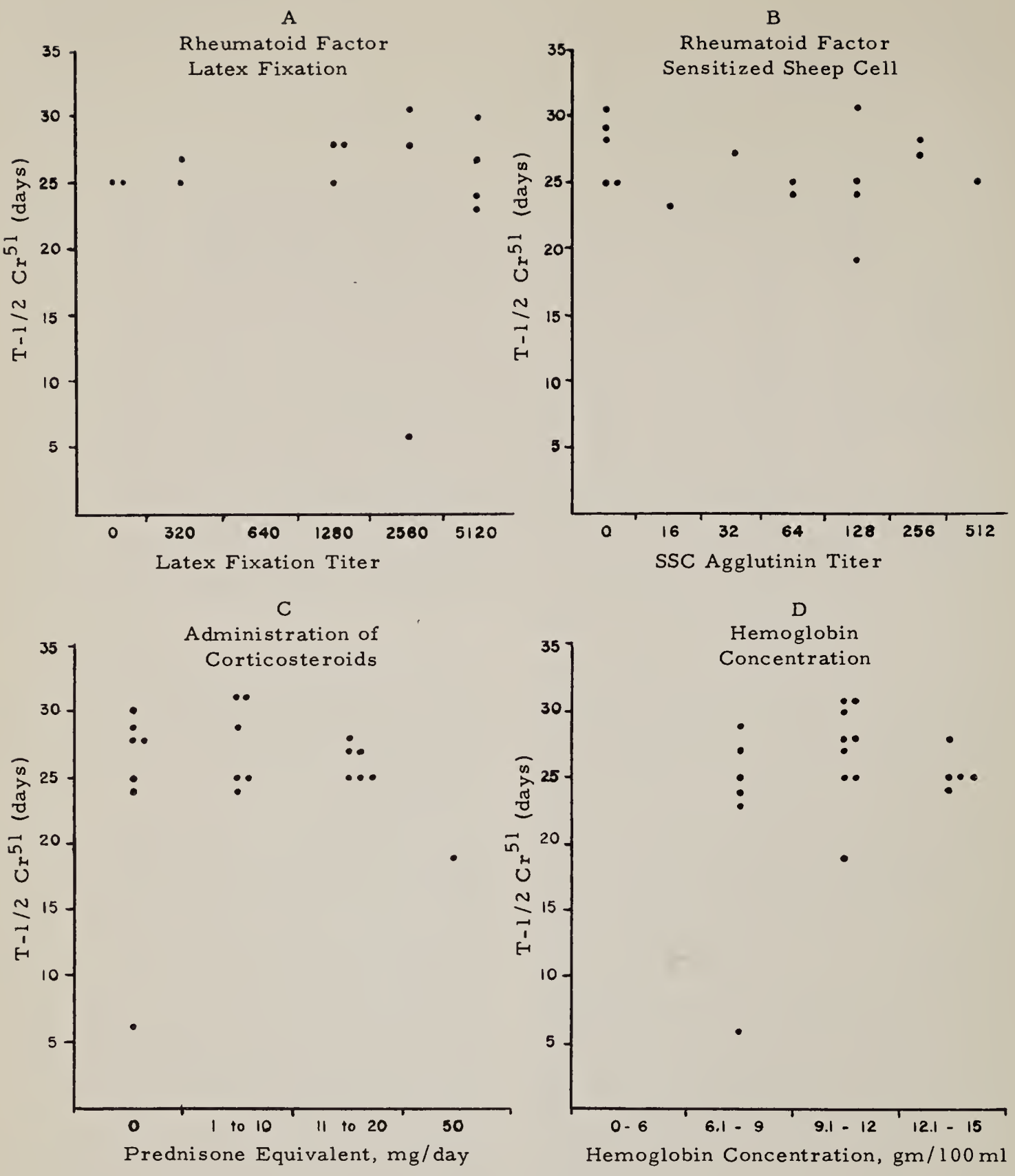

Fig. 2—Erythrocyte survival time in patients with rheumatoid arthritis as compared to other measurements.

If the arthritic patient is more than moderately anemic, a careful search for blood loss or other causes of anemia should be made. Available information[10] indicates that occult fecal blood loss from salicylate medication may be such a source, and the implications of concomitant peptic ulcer disease are obvious.

In connection with the thought that some manifestations of rheumatoid arthritis are the expression of autoimmune disease, we note that the red blood cell survival was not influenced by presence or absence or titer of rheumatoid factor or administration of corticosteroid drugs in our patients with rheumatoid arthritis. Similar conclusions apply, without generalizations, to the patient with systemic lupus erythematosus who presented for years as progressive polyarthritis, and to the two spondylitics studied.

For correction of the anemia, basic treatment of the underlying rheumatic disease is paramount. In such a program, iron therapy would seem indicated only if an individual arthritic patient can be shown additionally to be iron deficient. The most important factor in establishing this is absence of stainable iron in the bone marrow.

SUMMARY

Hematologic measurements including estimates of the red cell mass, plasma volume and red blood cell survival time have been performed on 22 patients, 19 having rheumatoid arthritis, 2 ankylosing spondylitis and 1 systemic lupus erythematosus. Anemia, if present, was mild, normocytic and hypochromic. The red cell mass was normal. The plasma volume tended to be increased. The red cell survival was usually within normal limits.

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THE NEURAMINIDASES OF INFLUENZA VIRUS

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The neuraminidases (sialidases) are enzymes of diverse biological origin which selectively remove a terminal sialic acid from various glycoproteins, oligosaccharides and mucolipids. Neuraminidase activity was first observed in the influenza virus[1] and subsequently shown to be associated with other viruses of the myxo group[2-4], with a variety of microorganisms[5-9], with commercial preparations of bovine and human glycoproteins[10] and with the chick chorioallantoic membrane[11].

There are two independent reports[12,13] of the crystallization of the neuraminidase from culture filtrates of *Vibrio cholerae* and we have separated the enzymes from several strains of influenza virus[14-16] and have recently effected their crystallization[17]. The neuraminidases have been described as α-O-glycosidases, cleaving the α-ketosidic linkage between the potential keto group of a terminal N-acylated neuraminic acid derivative and an adjacent sugar residue[4,18]. Substituted neuraminic acids are known collectively as sialic acids, and N-acetyl, N-glycolyl, O,N-diacetyl and N-acetyl-O-diacetyl substituted neuraminic acids have been described. The structure of the well-characterized trisaccharide, N-acetylneuraminlactose (NAN-LAC)* is given in Fig. 1. This compound is quantitatively cleaved at the α-glycosidic linkage by the neuraminidases to yield N-acetylneuraminic acid (NANA)and lactose.

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This work was supported by grants from the National Institutes of Health (E 4435) and from the Commission on Influenza, Armed Forces Epidemiological Board.

Properties of the Neuraminidases

Table 1 gives the comparative kinetic data we have obtained for the Asian and PR 8 enzymes and intact Asian and PR 8 viruses[16]. Also included are the results obtained by other workers for Asian virus[18], Lee virus[20], Lee enzyme and virus[21], the *V. cholerae* enzyme[12] and the enzyme from chick chorioallantoic membrane respectively[11]. It is clear that there are significant differences in the properties of the various enzymes. The pH optimum for the substrate NAN-LAC varies from 4.5 to 7.0, and the pH

*The following abbreviations are used: NAN-LAC, N-acetylneuraminlactose; NAN, N-acetylneuraminic acid

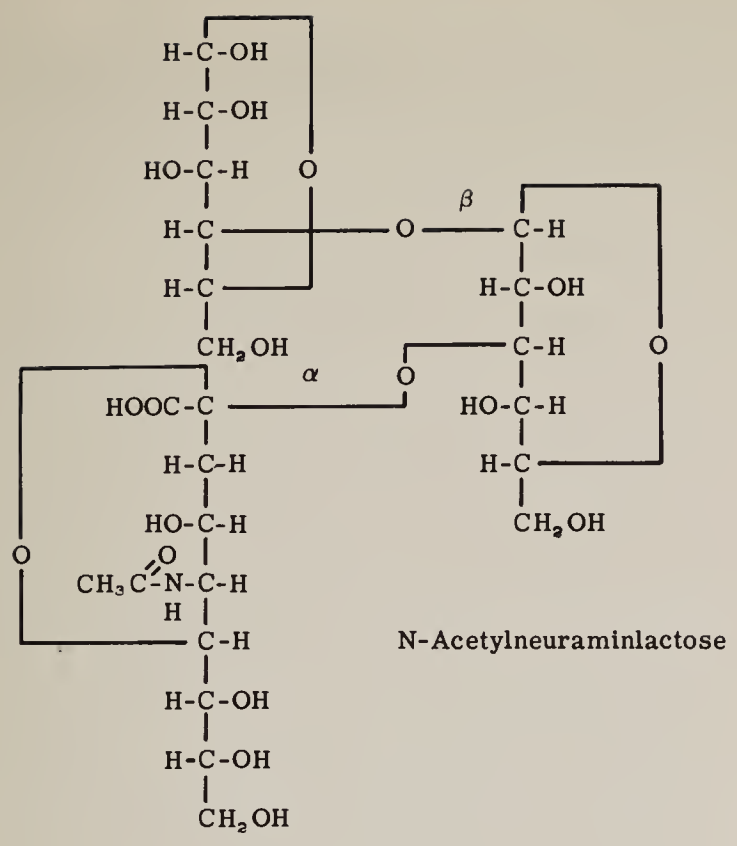

Fig. 1.—Structure of N-Acetylneuraminlactose (NAN-LAC).

optimum of a given enzyme may vary with the substrate employed. This latter point is documented for the PR 8 preparations and was previously reported for the Vibrio enzyme[13]. The Michaelis constants (K_M) also vary, indicating that the enzymes have different affinities for the substrate. There is a significant difference in the inhibitor constants (K_I) for the Asian and PR 8 preparations, indicating again differences in the affinities of the enzymes for a product of the reaction. The observation that NANA competitively inhibits the enzymic reaction, an observation first made by Walop[22], suggests that NANA may combine with the same site on the enzyme surface as does NAN-LAC and that it might be possible to demonstrate the transfer of NANA to some acceptor other than water. However, attempts to demonstrate the incorporation of radioactive lactose into NAN-LAC or to demonstrate transglycosidation reactions have not as yet been successful[23]. From the K_M and K_I values it may be concluded that the glycosidic bond in NAN-LAC increases its affinity for the enzyme some 5-fold over that of NANA. Neither the viral enzymes nor the neuraminidase from *Clostridium perfringens*[24] are stimulated by the addition of Ca ion, whereas the Vibrio enzyme[12] and the mammalian enzyme have increased activity in the presence of Ca. The viral enzyme[14], the mammalian enzyme[10] and the Vibrio enzyme of Ada *et al.* are inhibited by versene. The Clostridium enzyme[25] and the chick chorioallantoic membrane enzyme[11] are not susceptible to versene. Versene inhibits the viral enzymes in the absence of added Ca ion but not in its presence[23]. Similar results were obtained by Ada *et al.* for the Vibrio enzyme and may, as they suggested, indicate that the enzymes as isolated contain a metal ion as an essential component.

TABLE I

COMPARISON OF KINETIC PROPERTIES OF NEURAMINIDASES

Preparation	Ref.	NAN-LAC pH optimum	NAN-LAC K_M	NANA K_I	Stimulated by Ca Ion
Asian Enzyme	(16)	6.5 (6.5)*	5×10^{-4}	4×10^{-3}	No
PR 8 Enzyme	(16)	7.0 (5.4)*	1×10^{-3}	2×10^{-2}	No
Asian Virus	(16)	6.6 (6.5)*	6×10^{-4}	4×10^{-3}	No
PR 8 Virus	(16)	7.0 (5.5)*	3×10^{-3}	2×10^{-2}	No
Asian Virus	(19)	6.8	6×10^{-4}	5×10^{-3}	—
Lee Virus	(20)	6.8	3×10^{-4}	—	—
Lee Virus	(21)	—	2×10^{-4}	—	—
Lee Enzyme	(21)	—	2×10^{-4}	—	—
V. cholerae Enzyme	(12)	5.6	1×10^{-3}	—	Yes
Membrane Enzyme	(11)	4.5	1×10^{-3}	—	—

*pH optimum with orosomucoid.

It is essential to establish the relationship, if any, of the viral enzymes to the neuraminidase of chick chorioallantoic membrane as influenza viruses are routinely produced in embryonated eggs. The viral enzymes, which appear to differ from each other in their kinetic and immunologic properties (see below), also differ in their properties from those reported for the membrane enzyme. However, a careful comparative study of the physical, enzymic and immunologic properties of these enzymes is necessary before final conclusions should be drawn as to identity or lack of identity.

Some preliminary studies had indicated that the Asian enzyme preparations could elicit antibody formation in guinea pigs.* We have recently extended this work and have immunized guinea pigs with highly purified, although not crystalline, preparations of the Asian and PR 8 enzymes[16]. The sera from animals injected with Asian neuraminidase reacted specifically with the Asian virus in the hemagglutination-inhibition test. There was no cross-reactions with PR 8 virus, influenza B viruses or PR 8 enzyme. The same was true for sera from animals immunized with PR 8 enzyme; such sera reacted only with PR 8 virus or enzyme. Thus, the viral neuraminidases appear to exhibit an immunologic type specificity. The observation that hemagglutinin antibody is produced by the enzymes is not easy to interpret as the enzyme preparations neither agglutinate red blood cells nor inhibit their agglutination by added indicator virus[14,15]. The enzyme preparations used for immunization, although highly active and free from demonstrable hemagglutinin, may.well have contained fragmented hemagglutinin components which were antigenic and yet not capable of effecting hemagglutination. Alternately, it may indicate that the enzymic and hemagglutination sites of the virus are identical. While we do not favor this latter view[14], it is clear that further work is necessary to distinguish between these and other possibilities.

We conclude from the kinetic and immunologic results that the Asian and PR 8 enzymes are specific entities and may be considered, along with the neuraminidases from other sources, as members of an isodynamic group of enzymes which catalize the same reaction but differ in a number of properties.

Although a variety of compounds have been used as substrates for the various neuraminidases, we routinely employ the trisaccharide, NAN-LAC as the one of choice. NAN-LAC is relatively easy to prepare in a highly purified form[26], and it is cleaved quantitatively by the viral enzymes. In Table 2 are given the relative rates of liberation of sialic acid from NAN-LAC and various glycoproteins by the Asian enzyme at pH 6.5[23]. A constant amount of enzyme, assayed against NAN-LAC, was used and all substrates were present in concentrations to give the same sialic acid content. There are considerable differences in both the rate and extent of liberation of sialic acid from the various substrates. Whether the failure of the enzyme to remove all the sialic acid from various of the substrates is due to steric factors or to a difference in the linkage of a portion of the sialic acid or both is not known. The sialic acid residues are presumably terminal in all these compounds. The relative sequence observed may be accurate only for NAN-LAC, haptoglobin and orosomucoid as these were the only compounds tested at a pH (6.5) known to be optimal for their splitting by the Asian enzyme. It would be of interest to determine whether different neuraminidases release varying amounts of sialic acid from diverse substrates and what the relative rates of liberation and the results of sequential action of different enzymes might be.

There is no absolute specificity for the nature of the neuraminic acid derivative in compounds susceptible to the action of neuraminidases. Substrates are known which contain N-acetyl, N-glycolyl, O,N-diacetyl and N-acetyl-O-diacetyl neuraminic acids[4]. The susceptible linkages so far demonstrated are the 2→3 type in NAN-LAC and the 2→6 type in 6-(N-acetylneuraminyl)-N-acetylgalactosamine[4]. This 2→6 linkage is of interest

*Carried out by Dr. K. Jensen, Chas. Pfizer Co., Terre Haute, Indiana

as Kuhn[27,28] has reported the isolation of an isomer of NAN-LAC which is not cleaved by neuraminidase. The linkage in this isomer is from the 2 position of NANA to the 6 position of the galactose moiety of lactose. With regard to other types of linkage, some recent work by Kathan and Winzler[29] is of interest. They had earlier described[30] the preparation of an inhibitor of viral hemagglutination derived from human erythrocytes. This inhibitor is a glycoprotein containing 25% NANA, 12% hexose and 12% hexosamine. Treatment of the intact material with sodium borohydride does not result in the loss of any constituent. If, however, the glycoprotein is first treated with neuraminidase to remove the NANA, sodium borohydride reduction results in a loss of hexosamine equal to the amount of NANA removed. A linkage from the C_2 of NANA to the anomeric hydroxyl of the hexosamine is suggested by these results. If the conclusions are correct, than a linkage of the 2→1 type is also susceptible to neuraminidase action.

Colominic acid (poly NANA) is not acted upon by the Vibrio enzyme[4] nor by the viral enzymes (Table 2) but is cleaved by the enzyme from *C. perfringens*[25]. In the course of the investigation of the periodate oxidation of sialic acids and other carbohydrates, we had the occasion to study the periodate oxidation of colominic acid*[23]. The results lead to the conclusion that the linkages are 2→8, since 2 moles of periodate were utilized and one mole of formic acid produced per 14 moles of NANA. This corresponds to a molecular weight of approximately 4000 and is consistent with other estimates of size that have been made. The periodate consumption appears to be restricted to the cleavage of the bonds between C_7, C_8 and C_9 of the NANA residue on the non-reducing end of the colominic acid. Thus, the Clostridium enzyme has the ability to cleave what may be a 2→8 bond.

Some workers[31,32] have reported the isolation of nucleotide-oligosaccharides containing sialic acid. The compounds

TABLE 2

LIBERATION OF SIALIC ACID BY ASIAN NEURAMINIDASE

Substrate	Relative Rates	Per cent of Total Sialic Acid Liberated
NAN-LAC	100	100
Collocalia mucoid	65	85
Bovine submaxillary mucin	60	75
Haptoglobin 1.1	59	70
orosomucoid	42	67
stromal inhibitor[29]	25	57
uromucoid	13	75
Colominic acid	0	0

of this group contain equimolar quantities of uridine diphosphate, N-acetylglucosamine, galactose and sialic acid. The sialic acid may be removed enzymatically to yield nucleotide disaccharides. The N-acetylglucosamine is glycosidically bound to the phosphate group and the galactose residues are joined to the N-acetylglucosamine at the 1→4 and 1→6 positions, the 1→3 isomer not being detected. The position of attachment of the sialic acid residues is not known.

Action of Neuraminidases on Biologically Active Glycoproteins

Glycoproteins may be defined as substances containing oligosaccharides and peptides covalently bonded to give high molecular weight compounds. While the total carbohydrate content may vary considerably, all such compounds will contain hexose, hexosamine, a sialic acid and little or no uronic acid. Table 3 summarizes the results obtained when various serum glycoproteins were treated with neuraminidase. Three general classes of biologically active glycoproteins are considered here: 1) the specific binding proteins, 2) the erythropoietic and gonadotrophic hormones, and 3) the blood group substances.

With the exception of the corticosteroid-binding protein[38], removal of the sialic acid had no effect on the abilities of haptoglobin[33,34] the transferrins[35] or the thyroxine-binding protein[36,37] to com-

*Kindly provided by Professor S. B. Binkley.

bine with the appropriate substances. It is of interest that the strongly acidic carboxyl group (pK_a 2.4) of sialic acid is not involved in the binding processes. Since the pH optimum and the total peroxidase activity of the neuraminidase-treated and the untreated haptoglobin-hemoglobin complex, and the complex formed from neuraminidase-treated haptoglobin were all identical, it was concluded also that NANA was not involved in the peroxidase activity of the complex[34].

The biological activities of ertythropoietin[39], a highly purified and homogeneous preparation of gonadotropin from pregnant mares' serum[40], a menopausal gonadotropin[41] and FSH[42] were all destroyed by enzymic removal of the sialic acid.

The sialomucopolysaccharide isolated from ovarian-cyst fluid has both blood-group Le^a and virus-receptor activity[43]. The material contained 18% sialic acid which could be removed by neuraminidase. The removal of the sialic acid left a mucopolysaccharide which did not inhibit viral hemagglutination but was fully active as a Le^a substance. This may be taken an as example of a macromolecule which has two biological activities, each of which is associated with a different chemical structure. It has been shown that enzymic removal of sialic acid inactivated erythrocyte agglutinogens M and N, Lu^a and Lu^b, but not some 17 additional human blood group agglutinogens including S and s and M^g which is an allele to M and N[44,45].

It is clear from the brief consideration given above that the terminal sialic acid (there is no evidence for terminal sugars other than sialic acid in these compounds) can determine the biological activity in some instances. In other cases it does not appear to be involved and we must look elsewhere for our understanding.

The Role of the Influenza Virus Neuraminidase

The finding that influenza virus and other viruses of the myxo group possessed enzymic activity has perhaps stimulated more speculation than experimentation. This is understandable as we have had little knowledge until recently on the structure of the viruses or on the prop-

TABLE 3

THE EFFECT OF NEURAMINIDASE ON CERTAIN BIOLOGICALLY ACTIVE GLYCOPROTEINS

Preparation	Biological Activity	Ref.
haptoglobin	Combines with hemoglobin as usual; pH optimum and total peroxidase activity of complex unchanged	33, 34
transferrins	Iron-binding unaffected	35
thyroxine-binding protein	Thyroxine-binding unaffected	36, 37
corticosteroid-binding protein	Binding capacity destroyed	38
erythropoietin	Activity destroyed	39
pregnant mares' serum gonadotropin	Activity destroyed	40
menopausal gonadotropin	Activity destroyed	41
FSH	Activity destroyed	42
Sialomucopolysaccharide	Le^a Blood group activity unaffected but virus hemagglutination inhibition destroyed.	43
agglutinogens M & N and Lu^a & Lu^b	Blood group activity destroyed	44, 45
17 additional blood group agglutinogens	Blood group activity unaffected	44, 45

erties of the enzymes, and definitive experiments have been difficult if not impossible to design and carry out. It has been suggested that the enzyme promotes the entrance of the virus into the susceptible cell or that the enzyme breaks down an extracellular glycoprotein barrier. It has also been proposed that the enzyme is involved in the release of newly formed virus from the infected cell. There has been speculation[46,47] that the enzyme is involved in the synthesis of new viral material in the mucoid layer of the cell. This was based on the observation that the level of glycoprotein substrate in the chorioallantoic membrane of the chick embryo was related to the periodicity of viral multiplication, and to earlier observations that influenza virus contains a relatively high amount of host cellular material. The evidence is not compelling in favor of any of these views and final decisions must await further experimentation. A specific inhibitor for the viral neuraminidases could conceivably be of considerable use in elucidating the role of the enzyme in the infectious cycle of the virus.

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ABSTRACTS AND REVIEWS

OF PUBLICATIONS BY THE STAFF

The Role of Corticosteroids in the Treatment of Malignant Lymphomas. S. Kofman, C. P. Perlia, E. Boesen, R. Eisenstein, and Taylor, S. G., III: *Cancer, 15:338-345, 1962.*

Fifty-three patients with systemic malignant lymphomas were treated with corticosteroids. Many were refractory to radiation and/or alkylating agents and were in an advanced state of disease. Worthwhile regressions occurred in 28 patients. Regressions appeared to be more frequent and longer in patients with a good performance status and in patients who had had no previous therapy. Regressions were seen in patients with lymphosarcoma, Hodgkin's disease, and reticulum cell sarcoma, but were more frequent and more prolonged in patients with lymphosarcoma. We now have several different types of compounds (e.g., alkylating agents, antimetabolites, and corticosteroids) which are effective in the treatment of patients with systemic malignant lymphomas, and the intelligent use of all of these compounds should result in improved palliation. Radiation therapy was a valuable adjunct in the treatment of localized complications in some of these patients.

Factors Influencing Leak of Perfusate from Isolated Limb Perfusion. D. M. Buckner, J. D. Ellenby, G. Messmer, J. Busch and S. G. Economou, *A.M.A. Arch. Surg. Oct., 1961.*

In 1958 Creech and co-workers described a method of treating cancer by regional and isolated perfusion. The object was to deliver a greater dosage of anti-cancer agent to the tumor bearing area without getting a high systemic dose of drug and the concomitant side effects of depressed bone marrow function, nausea, vomiting, anorexia and etc.

It was found early, however, that there was some leakage of perfusate from the treatment area during the perfusion to the general circulation and thus side effects were experienced which tended to defeat the purpose of the technique.

This project was concerned with determining the factors involved in leakage of perfusate containing RISA (Dextran 6% in normal saline) from the isolated hind limb of the rabbit to the systemic circulation and attempts were made to decrease leakage by a variety of methods. Low flow rate, cooling of perfusate and patent venous return flow all tended to minimize leakage. Addition of anticoagulants or antispasmodics increased leakage. Time in and of itself was not detrimental.

Status Report on Tissue-Culture Cultivated Hepatitis Virus. I. virology laboratory studies—W. A. Rightsel, R. A. Keltsch, A. R. Taylor, Detroit; J. D. Boggs, Chicago and I. W. McLean, Jr., Detroit. II. clinical trials—J. D. Boggs, R. B. Capps*, Chicago; C. F. Weiss, Ann Arbor, Michigan; and I. Wm. McLean, Jr., Detroit.

**Staff member*

J.A.M.A.: Vol. 177; pp. 671-682, 1961.

A tissue culture system is described which permits identification and propagation of isolates from the blood of hepatitis patients. Techniques first described in 1956 have been greatly improved. Viruses thus isolated produced hepatitis in 3 clinical studies. Incubation period, symptoms, clinical manifestations, and laboratory findings were typical of infectious hepatitis. Viruses were consistently reisolated from these cases and specific neutralizing antibodies were demonstrated. Limited studies indicated at least 3 distinct virus serotypes, 2 of which produced hepatitis with jaundice. Application of ultrafiltration, ultracentrifugation, and electron microscopy to infectious tissue culture fluid revealed 12 to 18 mμ particles not found in controls. Similar particles were noted in infected cultured cells. Despite difficulties, these clinically proved laboratory techniques insured continued characterization of hepatitis viruses. These techniques may eventually become tools for diagnosis and preparation of antigens and vaccines.

Recurrence at the Suture Line Following Resection for Carcinoma of the Colon—Incidence Following Preventive Measures. H. W. Southwick, W. H. Harridge, and W. H. Cole. *American Journal of Surgery, Vol. 103, No. 1, January, 1962, pp. 86-89.*

A preliminary survey of the cause of treatment failure following anterior resection for carcinoma of the colon in 1951 revealed that 10.9 per cent of the patients developed recurrence at the anastomotic suture line. A more extended review covering the period from 1936 to 1954 confirmed the fact that this suture line recurrence presented a significant problem.

In an effort to combat this potential local implantation as the etiologic factor of these recurrences, certain prophylactic measures were instituted in 1954 at the University of Illinois Research and Educational Hospitals. Prophylactic measures consisted of preliminary ligation of the bowel several centimeters proximal and distal to the tumor, excision of the tissue crushed by the clamps preliminary to the division of the bowel, and irrigation of the bowel before performance in the anastomosis were employed in 101 patients over the five year period from 1954 through 1959.

In this group of patients, all of whom have been followed for at least one year, there have been no recurrences at the suture line in the treatment failures.

Separation of Adenine, Hypoxanthine, and the 5,-(Mono-, Di-, and Tri-)-Phosphates of Adenosine and Inosine by One-Dimensional Paper Chromatography. A. Aras, M. Becker, A. Brown and G. Hass. *Lab. Invest., 11:65-69, 1962.*

A method has been described for separation of adenosine and inosine and their 5'-(mono-, di-, and tri-)-phosphates, as well as adenine and hypoxanthine, by one-dimensional paper chromatography in 24 hours at 32 ± 2°C. on S&S No. 589 Orange Ribbon filter paper, with an isobutyric acid-1 M NH_4OH-5 N NaOH-0.1 M Na_3EDTA (pH 9.5)-tetrahydrofurfuryl alcohol (100:50:7.5:2.5:40) solvent. The amounts which may be detected and separated are of the order of 2 to 20 μg.

This method has proved useful in the determination of small amounts of products which have appeared in certain substrates exposed to the enzymatic action of isolated contractile myofibrils from human cardiac and skeletal muscle. As a result, some distinctions have been drawn between myofibrils prepared from the heart affected by different diseases. These distinctions refer to differences in enzymatic activities.

1962 Poison Control Program in Chicago. J. R. Christian. *Chicago Medicine, Vol. 64 No. 38, March 17, 1962.*

On April 1, 1953, the first formal meeting of the Poison Control Committee of the Illinois Chapter of the American Academy of Pediatrics was held in Chicago under the chairmanship of Dr. Edward Press. A model for poison control centers was designed and as a result such centers have been established in all states and most communities in the United States, as well as throughout the world.

During the intervening years, service to patients and practical experience have helped develop methods of education for medical, para-medical personnel and the public. Research programs have been developed and revised, as well as new concepts of therapy, have resulted. Eleven Chicago hospitals participated as referral and treatment centers. The remaining 77 Chicago hospitals with emergency room services have been utilized as emergency treatment centers. At the end of 1961, the Poison Control Advisory Committee reconsidered and re-evaluated the Poison Control Center and the Chicago Program. Under the decentralized program, sufficient progress had not been made during the past nine years. Therefore, the members of the Poison Control Advisory Committee recommended that a master poison control center for the City of Chicago be planned and that the center should be developed by Dr. Joseph R. Christian at Presbyterian-St. Luke's Hospital.

The Poison Control Center at Presbyterian-St. Luke's Hospital functions as follows:

1. A 24 hour a day poison information service under the direction of Louis Gdalman, Director of Pharmacy Services Department. Consultants to the center are available.
2. Whenever possible, sources and availability of antidotes are listed among reference materials.
3. Adequate records of calls received for information are kept.
4. The center acts as a stimulus for an educational program.
5. Cases treated in the Poison Control Center are reported on forms provided for this purpose.
6. Emergency Personnel are adequately orientated in the treatment of poisoning cases and operating procedures are established for 24 hour a day treatment.

Future planning has been directed toward establishment, development and staffing of toxicologic and treatment facilities. Utilization of medical and surgical specialties such as the Renal Laboratory and the Artificial Kidney, Pathology, Biochemistry, Intensive Patient Care Unit, etc. will be synchronized with establishment of toxicologic and treatment facilities so that care, as well as research, professional and lay education can result.

Topical Treatment with Antirheumatic Agents. E. F. Traut and C. F. Thrift, *Ill. Med. Jour. 121 p. 257 March, 1962.*

To rub a painful part of one's body is instinctive. Such stroking, formalized, is massage, a universally recognized treatment of many rheumatic conditions. Chemicals are added to massage in liniments or ointments to produce the sensation of heat and relieve pain.

In spite of wide popular use, advertisement in journals and recommendation in clinical tests almost no studies of their mode of action or clinical effectiveness exist.

The benefits of liniments are irritation of a rubefacient together with massage aimed at obtaining the absorption of salicylate.

We administered successively a perfumed salve as placebo (A) and an indistinguishable ointment (B) containing esters of salicylic, nicotinic and benzoic acids, to 146 patients. Ninety-eight of these had degenerative arthritis; 25 rheumatoid arthritis; 7 patients "primary" fibrositis; 3 gouty arthritis and 1 psoriatic arthritis. Negro females outnumbered other patients 3 to 1. All patients were first evaluated with oral and injected placebos. All had previously been subjected to almost all known forms of antirheumatic therapy.

The ointments were rubbed into an area on one side, using a correspondingly involved area as a control.

Successes or failures were judged by relief of pain, stiffness, swelling and tenderness. Either or both ointments were judged successful in 142 patients. Both preparations failed in 4. A was helpful subjectively, objectively or both in 142 patients. B gave more relief than the medicated ointment in 27 patients. Both preparations were equally beneficial to 33 patients. Patients with the least evidence of disease derived the most relief. Eighty-three patients emphatically and enthusiastically reported most benefit from B. Relief was felt progressively during the rubbing-in of the ointment and lasted about four hours, rarely weeks. The superior effects of B over A approximate 3:1. Patients admitting no benefit from either ointment were 4 white males, possibly the most critical group.

Transient rashes following the use of A as well as B were the only side-effects.

Of the 142 patients benefiting from the ointments 4 claimed to have had more relief from placebo tablets. All 142 had been more relieved by placebo injections than by the ointments. All of the 142 patients wanted to continue the ointment with their previous treatment.

PATIENTS REPORTING	146
Medicated Ointment—Superior	83
Placebo Ointment—Superior	27
Both Ointments—Equal	33
Neither Ointment—Effective	4

The Neurogenic Factor in Raynaud's Phenomenon. G. de Takats and E. F. Fowler. *Surgery, 51: 1, 9-16, Jan. 1962.*

A group of 66 patients exhibiting Raynaud's phenomena were followed from one to 25 years. It has been assumed in the past, that the variable and unpredictable results of dorsal sympathectomy were overwhelmingly due to an inability to sympathectomize an upper

extremity completely, and to a high incidence of regeneration. Of 424 patients collected from the literature and including our own, 35% were regarded as not having benefited from the operation.

It is the authors' thesis that the poor results were due to poor case selection. Their observations show that when Raynaud's phenomena are due to organic vascular disease or to increased central sympathetic discharge, the results of sympathectomy were excellent. Should the phenomena be due to incipient or manifest collagen disease or to an abnormal immune reaction of the blood constituents to cold, sympathectomy can not help. A systematic employment of diagnostic methods, including the determination of the critical temperature at which vasospasm occurs, the emotional response, digital biopsy, arteriogram, digital plethysmography, electroencephalography and the study of cold agglutinins, cryoglobulins and slit lamp study of conjunctival vessels has been utilized to separate the various factors responsible for Raynaud's phenomena.

Enzyme Activity in the Nephron: Quantitive Study of Ultramicrochemical Techniques. V. E. Pollak and H. Mattenheimer. *Arch. Int. Med. 109:473-484, 1962.*

The ultramicro biochemical techniques used to study enzyme activity in the various portions of the nephron dissected from frozen dried kidney sections are reviewed in detail. It was shown; (1) that it was possible to dissect out accurately the individual portions of the nephron. (2) That the average coefficient of variation for weighing on a quartz fiber "Fish-pole" balance with a range of 10 to 100 mμg was 1.1 per cent. (3) That the coefficient of variation for the measurement of enzyme activity in homogenates varied from 1.9 to 2.9 per cent, indicating that the ultramicro techniques were as accurate as macrotechniques. (4) That the biologic variation in enzyme activity from nephron to nephron in the same kidney was between 0 and 20 per cent. (5) That for some enzymes there is a considerable variation in activity from one animal to another in the same species whereas for others there was little variation.

Endoscopic Surgery of Zenker's Diverticula—Experience with the Dohlman Technique. P. H. Holinger and K. C. Johnston. *Annals of Otol., Rhin., and Laryng. 70:1117-1123, Dec. 1961*

The Dohlman method of management of the pharyngo-esophageal (Zenker's) diverticulum consists of the endoscopic exposure and sectioning of the cricopharyngeus muscle. The operation is performed by means of a specially designed endoscope, cauterizing forceps and knife. Under intratracheal anesthesia the double-bladed endoscope is introduced into the pharynx; the anterior blade is guided into the esophagus and the posterior blade into the sac, engaging the cricopharyngeus muscle in a U-shaped slit between the blades. The view through the endoscope at this time shows the upper edge of the smooth, rolled mucosal wall that separates sac and esophagus. This is grasped in the center by an alligator-type forceps, cauterized and cut. The incision is carried close to the base of the sac, in effect eliminating the party wall between diverticulum and esophagus. Eleven patients (now fourteen) with this type of diverticulum have been operated using this endoscopic approach. There have been no post-operative complications and all have been relieved of their cough, dysphagia and regurgitation. The short duration of the operation and the post-operative hospitalization are of special benefit in the poor surgical risk, elderly patients.

A Psychiatric Study of Patients with Psychomotor Seizures, R. D. Chessick and R. R. Bolin, *J. Nerv. and Ment. Dis., 134:72, Jan., 1962.*

A number of characteristic features were found in a study of the life history and personality of eleven patients with "psychomotor epilepsy." In brief, there were: a traumatic early history; continuing struggle with critical unresolved conflicts centering around dependency needs and wishes; serious problems with easily aroused rage, and with destructive impulses which were poorly integrated; excessive use of repressive defenses which resulted in an impoverishment of overall ego functioning, together with a readiness for the use of the more primitive psychologic defenses of denial and projection; lability of affect; an overall physiologic and psychologic rigidity manifest in everyday thinking and acting.

The Kidney in Rheumatoid Arthritis: Studies by Renal Biopsy. V. E. Pollak, C. L. Pirani, I. E. Steck, and R. M. Kark, *Arthritis and Rheumatism, 5:1-9, 1962.*

Renal biopsies and tests of renal function were made on 41 selected patients with rheumatoid arthritis. The patients were chosen for study because it was thought that these patients were most likely to have renal abnormalities.

No histologic abnormality was found in 21 cases. Arterio- and arteriolosclerosis or arteriolar nephrosclerosis was found in 14 biopsies. Amyloidosis occurred in 4 biopsies, and lupus nephritis was diagnosed in 2. Glomerulitis of the type described by others in postmortem studies was not observed.

Proteinuria was observed persistently in 13 patients. It was associated with amyloidosis in 4, with lupus nephritis in 2, and with renal vascular disease in 4. In 3 patients no underlying histologic abnormality was found. Tests of renal function were within normal limits in almost all the patients. Slight elevation of the serum urea nitrogen level was found in only 5, 2 of whom had renal amyloidosis and 2 had arteriolar nephrosclerosis.

Eighteen patients had been treated with gold salts. Although proteinuria first appeared in one patient after treatment with gold salts was started, in the series as a whole proteinuria occurred as frequently in those who had never received gold therapy as in those who were being treated with gold. We found no histologic evidence for a toxic effect of gold salts on the kidney except for the presence of slight fatty metamorphosis in the convoluted tubules of one patient.

Multiple Sclerosis and Pregnancy. J. S. Garvin. *Am. J. of Obst., and Gynecol. 83:689-693, March 1, 1962.*

Neurologists long have held that multiple sclerosis is adversely affected by pregnancy. A case is presented to illustrate the problem—the woman, a 37 year old gravida iv, para iii anxious to have another child, developed symptoms of multiple sclerosis for the first time at age 31 during the second month of her fourth pregnancy with partial loss of vision in the right eye, migratory paresthesias and slight ataxia. Pregnancy was allowed to continue, and symptoms ultimately disappeared. Transient exacerbations of symptoms without permanent deficit occurred three times within the next five years.

Recent studies, have indicated that there is little, if any, difference in the course of the disease in parous and nulliparous women, taking into consideration the fact that women with severe forms of the disease are less likely to marry and become pregnant.

No statistics are available to determine whether pregnancy will adversely affect the patient already disabled, or with progressive disease. Based on the experience of the author and of other neurologists, the impression is that pregnancy may adversely affect such a patient. Scattered reports of therapeutic abortion in the course of multiple sclerosis do not suggest that the procedure is of definite benefit.

Modern Plastic Surgical Treatment of Unilateral Facial Paralysis. J. W. Curtin, P. W. Greeley. *GP, Vol. XXV, No. 2, Feb. 1962.*

Unilateral facial paralysis is showing a definite increase in incidence. This increase is due to the rising automobile accident rate, associated with an increase in cases of severe facial trauma and a more radical surgical attack on extensive malignancies of the face. Regardless of its etiology, it causes disfigurement with associated psychic trauma that many people find distressing. Modern plastic surgery has much to offer these people in overcoming their facial deformities. Primary nerve suture or nerve graft must be considered in early cases following known division of the facial nerve. Others should be treated by elevation of the paralyzed side with autogenous strips of fascia lata. A lateral tarsorrhaphy gives best support to the lower eyelid. Selected cases must be chosen where sections of certain muscles of facial expression on the unaffected side are removed in order to cut down the excessive activity of the so-called "normal side". This will give the patient a more balanced bilateral facial expression.

Representative cases with case reports are demonstrated in the paper.

Quantitative Histochemistry of the Nephron. R. M. Kark, H. Mattenheimer, S. L. Bonting, V. E. Pollak and R. C. Muehrcke. *Ciba Symposium on Renal Biopsy. Wolstenholme, G. E. L., and Cameron, M. P., eds. London, J & A Churchill, 1961, pp. 309-324.*

This paper reviews briefly techniques used to study enzyme activity (ultromicro biochemical techniques) in the glomeruli, proximal convolutions, distal convolutions, medullary rays and medulla of healthy and diseased human kidneys. Preliminary results were given for glutamic oxalacetic transaminase, glutamic dehydrogenase and lactic dehydrogenase activities on the individual portions of the nephrons from three healthy kidneys and from a kidney of a patient with renal tubular acidosis. The activity of these enzymes was not different from normal in the case of renal tubular acidosis studied. Carbonic anhydrase activity was similar in the nephrons of one healthy kidney and one case of renal tubular acidosis, suggesting that carbonic anhydrase deficiency is not a rate limiting factor in the failure of the patient with renal tubular acidosis to secrete hydrogen ion.

Perforated Peptic Ulcer. E. L. Strohl, F. W. Preston, R. J. Baker. *Illinois Med. Jour. 120:14-23, July, 1961.*

1. Acute perforation of a peptic ulcer through all coats of the stomach or duodenum allowing free escape of gastric or duodenal content into the peritoneal cavity is the most serious complication of peptic ulcer.
2. Free perforations occur primarily in males, the ratio being 25 or 30:1, males to females.
3. Free duodenal ulcer perforations occur four or five times more frequently than free perforation of a gastric ulcer. About 25 per cent of all acutely perforated ulcers have no history of previous symptoms.
4. "Walled-off" perforations, or penetrating ulcers comprise about one third of so-called "intractable" ulcers, those which are very resistant to medical treatment. They are usually on the posterior wall of the duodenum, or, along the lesser curvature on the posterior side of the stomach, and, are adherent to the liver, pancreas, or gastrohepatic omentum.
5. The symptoms of free perforation of a peptic ulcer are usually heralded by sudden onset of upper abdominal pain, which quickly becomes generalized. Nausea and vomiting usually follow the pain, and prostration is generally present.
6. Physical findings are, classically, those of a tender, rigid, silent abdomen; the patient resists change in position, preferring to lie flat and motionless. Tachycardia and normal or slightly elevated temperature are the rule; shock is rarely present until late in the course of the disease.
7. Approximately 80 to85 per cent of perforated peptic ulcers will show varying amounts of free air on one of the usual "four views" of the abdomen.
8. Surgical treatment of perforated peptic ulcer is the treatment of choice in almost all patients, particularly those perforations less than 24 hours old. The following procedures have been advocated:
   a. Simple omental graft
   b. Closure of the ulcer
   c. Excision of the ulcer and closure (with a pyloroplasty when necessary)
   d. Excision of the ulcer, pyloroplasty, and vagotomy
   e. Gastric resection

   Each has its place, but at this time, simple omental grafting with a free or living graft is the procedure of choice.
9. An extremely important aspect of the treatment of the acute perforation at the operating table is a thorough cleansing of the peritoneal cavity of gastric or duodenal content, food, etc., by meticulous aspiration of the peritoneal gutters, pelvis, and subphrenic spaces.

Surgical Physiology of Hypertension. G. de Takats, *The Surg. Clin. of N. Amer., 42: 1, Feb. 1962.*

There is an increasing number of hypertensives whose elevation of blood pressure is due to a known and remediable cause. Unilateral renal disease, pheochromocytoma, Cushing's syndrome, primary aldosteronism and coarctation of the aorta belong to this group of *secondary hypertensions*. But the bulk of hypertensives still belong to the category of *primary* (essential) hypertension of unknown etiology. The neurogenic factor is prominent in this group as attested by the increased reactivity to cold, to the inhalation of carbon dioxide and to emotional stimuli. The progression from an intermittent pressor response to a continuous hypertension involves a renal mechanism, a salt-retaining factor and a higher setting of the pressure-regulating receptors in the branches of the aorta. Any method that will bring about a distinct lowering a diastolic blood pressure will decelerate the progress of vascular damage. If this can be done with readily controllable and nontoxic medication, the surgeon need not be concerned. Surgical activity, however, should begin at a phase of drug-resistant hypertensive cardiovascular renal disease, when the damage can still be halted, nay, often reversed. Careful selection of patients for sympathectomy has brought about some striking results in a 10-22 year follow-up of 105 personally observed cases.

Lupus Nephritis. Ciba Symposium on Renal Biopsy. C. L. Pirani, V. E. Pollak, R. C. Muehrcke and R. M. Kark. *Wolstenholme, G.E.L. and Cameron, M. P., eds. London, J & A Churchill, 1961, pp. 309-324.*

Single or serial renal biopsies were studied in 105 patients with systemic lupus erythematosus (SLE). The renal involvement starts with local and focal proliferation of endothelial cells in the glomeruli (lupus glomerulitis) associated with thickening of the capillary basement membrane and fusion of epithelial foot processes. In the glomeruli of the more severe and active disease (lupus glomerulonephritis) there is necrosis with fibrinoid, hyaline thrombi, karyorrhexis, hematoxyphil bodies, "wire loop" lesions and basement membrane thickening. Until a late stage a local and focal distribution of the glomerular lesions is characteristic. As the glomerular lesions gradually become more diffuse and generalized their specific character becomes less apparent.

Symptomatic treatment with small doses of cortisone or prednisone, sufficient to control the general clinical symptoms of SLE, does not modify significantly the clinical and histological signs of lupus nephritis.

Prolonged treatment with large doses of prednisone appears to control the "active" lesions of lupus nephritis and to slow down the progression of the renal lesions. As a result the average life expectancy of these patients is significantly prolonged.

A Test of Connective Tissue State and Reactivity in Collagen Diseases. D. M. Laskin, M. B. Engel, N. R. Joseph and V. E. Pollak. *J. Clin. Investigation, 40:2153-2161, 1961.*

The rate at which connective tissue ground substance is disaggregated and restored after injection of hyaluronidase may be taken as an index of its initial state and its capacity for repair. This reaction was studied by an electrometric method involving the serial measurement of displaced Donnan potentials in the dermis. Normal persons and patients with rheumatoid arthritis, scleroderma and systemic lupus erythematosus were compared. The results indicate that in these connective tissue diseases a ground substance is formed which differs both qualitatively and quantitatively from the normal. The rate of reconstitution after enzymatic action is markedly delayed. The administration of corticosteroid tends to reverse these conditions. The measurements of changes in the state of the ground substance after the injection of hyaluronidase is a useful test for evaluating connective tissue diseases and the effects of therapy.

Human Bites of the Face Early Treatment and Late Reconstructive Repair. J. W. Curtin, P. W. Greeley. *J. Pl. & Reconst. Surg., 28: 4, Oct. 1961.*

Human bites of the face are almost always true bites and associated with the loss of soft tissue and cartilage. Because of the circumstances of injury the patient is reticent to come for

early treatment; thus many are seen days later with severe infections and eventual loss of important structures of the face.

Many hospital emergency rooms still post antiquated and barbaric methods of therapy including electrocoagulation or acid cautery of the wound.

Very few recent articles on the subject have appeared to bring the physician up-to-date on the proper care of these unfortunate injuries. Such is the purpose of this paper and in addition representative cases and methods of late reconstructive repair are entailed.

Gangrene and Perforation of the Gallbladder. E. L. Strohl, W. G. Diffenbaugh, J. H. Baker, M. H. Cheema. *Surg., Gynecol. & Obst. 114:p 1-8, Jan., 1962.*

Gangrene of the gallbladder is complete necrosis of a portion of the gallbladder wall, in one or more areas. The pathologic process is a sequela of acute calculous cholecystitis, and it is rarely encountered when no stones are present in the gallbladder. The pathologic changes leading to gangrene and perforation are usually the result of a stone having lodged in the cystic duct so that the outflow of bile is prevented. In addition, the gallbladder usually contains one or more stones, bile mixed with blood, colorless bile, or pus under pressure.

Perforation of the gallbladder as a sequel of acute cholecystitis may occur in one of the following ways: (a) into the free peritoneal cavity; (b) about adhesions into the gallbladder bed, forming a pericholecystic abscess; or (c) into adjacent hollow viscera, forming a fistulous tract.

In a review of the literature, reports of 7,708 patients with acute cholecystitis were encountered. In this group there were 647 patients with perforation of the gallbladder. This is an incidence of 8.3 per cent perforation in acute cholecystitis. The mortality rate in those patients who had perforation of the gallbladder was 19.0 per cent.

The average age of our patients with gangrene and perforation of the gallbladder was 60.8 years.

Patients with acute cholecystitis have pain, tenderness, and frequently a palpable mass in the right upper quadrant. These findings and symptoms are also those most frequently encountered in gangrene and perforation. The most helpful factor in the diagnosis is evidence of progression of the disease. Progression of the acute cholecystitis is manifested by a rise in the pulse rate, enlargement of the mass in the right upper quadrant, and a marked increase in the tenderness to palpation. The evidence is obtained only by careful and frequent observation of the patient.

Surgical Management of Internal Carotid Occlusion. H. Javid, and O. C. Julian. *American Heart Bulletin—1962.*

Clinical features of 195 patients with extra-cranial occlusive lesions of the internal carotid artery were reviewed and classified into four types.

1. Marked stenosis of the internal carotid artery with manifestations of intermittent cerebral ischemia.
2. Moderate stenosis of internal carotid artery producing embolic cerebral infarct.
3. Complete occlusion of internal carotid artery not amenable to surgical correction.
4. Tortuous and kinked internal carotid artery.

The ideal candidate for surgery is the patient with partial and segmental occlusion of the internal carotid artery. An excellent response was noted in 80% of patients treated in this group. An additional 8% had significant improvement. Great emphasis should be placed on diagnosis of symptomatic arterial stenosis. Correction of such a lesion in our opinion will prevent stroke.

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STUDIES IN ABNORMAL CALCIFICATION

Reuben Eisenstein, M.D.

One of the very common reactions of tissues to injury is the accumulation of calcium salts in areas of tissue damage. In tissues such as bones or teeth it is known that calcium is deposited extracellularly as crystals of hydroxyapatite and is located within collagen or dentine fibers. In recent years there has been some elegant work on bone which has begun to elucidate quite precisely the relationship between the organic matrix in which calcium salts are deposited (collagen in the case of bone) and the inorganic crystal phase[1]. In the case of calcification in tissues, which do not normally calcify, the situation is much less clear[2]. Even collagen, which normally calcifies in bone, is quite resistant to calcification elsewhere.

Any consideration of calcification must involve the two components of calcareous deposits, the salt itself and the organic matrix in which the salt is deposited. With some exceptions the salt is a calcium phosphate arranged in the form of an apatite crystal. If any mineral salt is to be deposited in the mammalian body, one could almost predict that it would be one of calcium and phosphorus, since calcium phosphate is relatively insoluble and the normal concentration of these ions in the body fluids is so high as to be considered as supersaturated[3]. However, there are obvious local factors which predispose to calcification, particularly in disease, for if this were not the case we would either be jellyfish or lithopedions.

Our interest in this problem has been related to the changes which predispose to abnormal calcification in tissues, the nature of the organic matrix in which mineral crystals are deposited and some local metabolic events which occur in calcified loci. One of the first questions to be asked is what comes first, the organic matrix or the mineral? In the special case of calcification associated with hypercalcemia, this now appears relatively clear from histochemical studies in other laboratories[4] and some chemical and isotopic studies here. In the rat almost all the radioactive sulfate which is converted to organic material is incorporated into acid mucopolysaccharides. Large amounts of these substances are present in cartilage and in some of the organic matrices which form in the kidney and calcify under the influence of large doses of vitamin D. Advantage was taken of this in rats given $Na_2S^{35}0_4$ and large doses of vitamin D. In these animals there was less radioactivity in cartilage and more in the serum, findings similar to those noted by others in hyperparathyroidism. This was expected, since doses of vitamin D of this magnitude are known to result in bone and cartilage destruction, and one would expect labelled material from cartilage matrix to appear in the blood. In the kidney, there was an increase in calcium before a calcified matrix could be seen histologically, and the amount of radioactivity did not increase until a calcified matrix was seen[5]. Further experiments along

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From the Division of Pathology

This work was supported by grants from the Otho S. A. Sprague Memorial Institute and United States Public Health Service (H-6713).

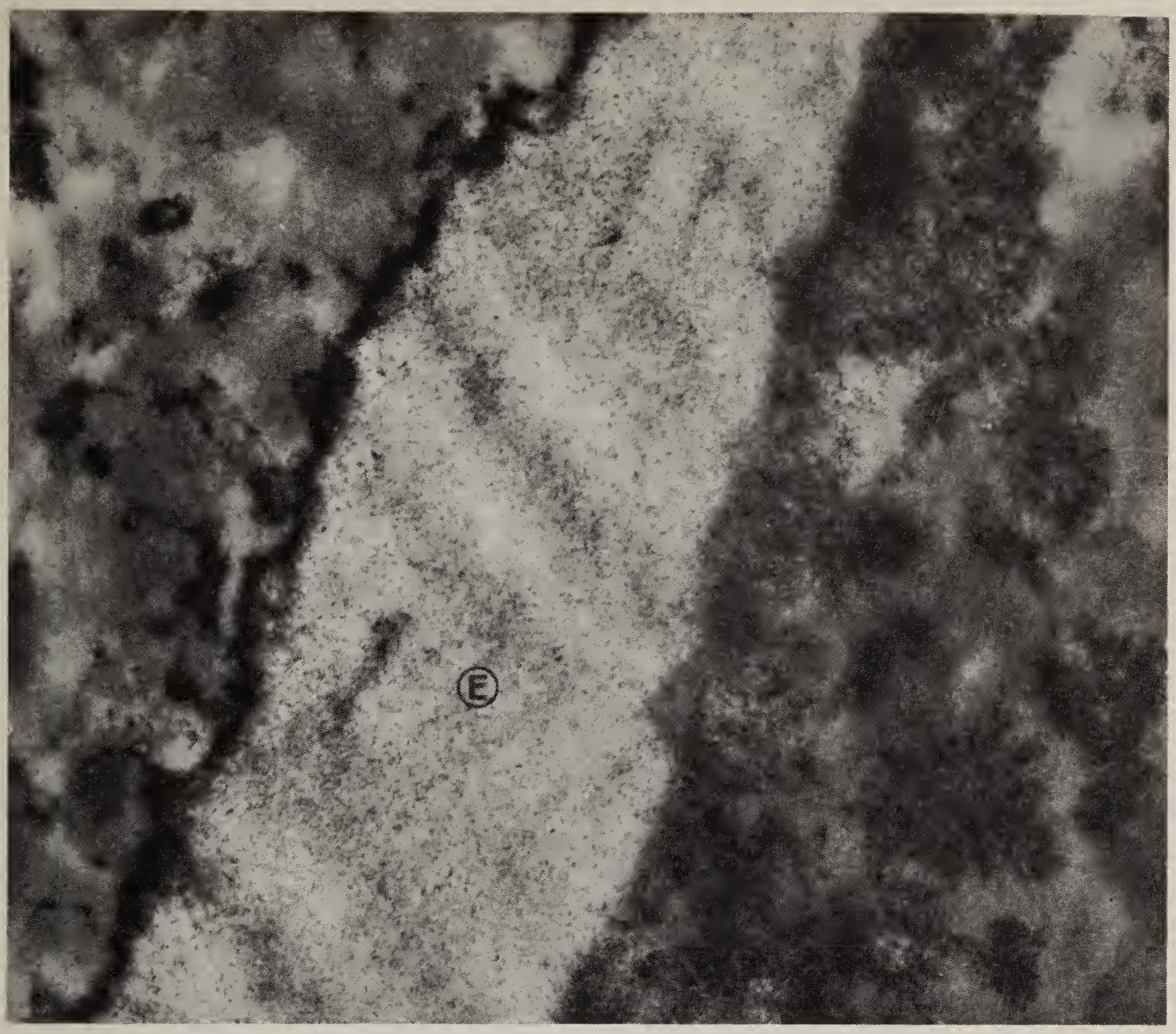

Fig. 1.—Aorta of rabbit with calcification induced by vitamin D. Small mineral crystallites are present within elastic fiber (E). In the surrounding non-fibrillar extracellular space, the crystals are larger.

this line indicate that the following sequence of chemical events occurs. Hypercalcemia occurs first. This is followed by an increase in calcium in the kidney and then a virtually simultaneous appearance of phosphate and an organic matrix which eventually contains and metabolizes acid mucopolysaccharides. It thus appears that hypercalcemia induces renal damage in such a way that the kidney accumulates calcium, which secondarily binds phosphate, which is followed by and perhaps stimulates formation of an organic matrix and that this matrix may stabilize the calcific deposit. The precise nature of the matrix, its mode of synthesis and the way it maintains the calcific deposit, as well as the nature of binding of the initial increment of calcium are unknown.

We are also studying a specific protein matrix which undergoes calcification in humans. The amount of calcium in the elastic tissue of human aortas, but not pulmonary arteries, increases with age. In an attempt to determine if this is due to a change in the elastic tissue itself, the maximal binding capacity of isolated human arterial elastic tissue for calcium was determined. It was found that elastic tissue from the aorta bound much more calcium than that from the pulmonary artery. Furthermore, binding of calcium by elastic tissue was directly correlated with age. Thus, beginning in adolescence, aortic elastic tissue bound progressively more calcium with increasing age until age 45, after which there was no further increase. Binding of calcium from human serum was quite substantial. The nature

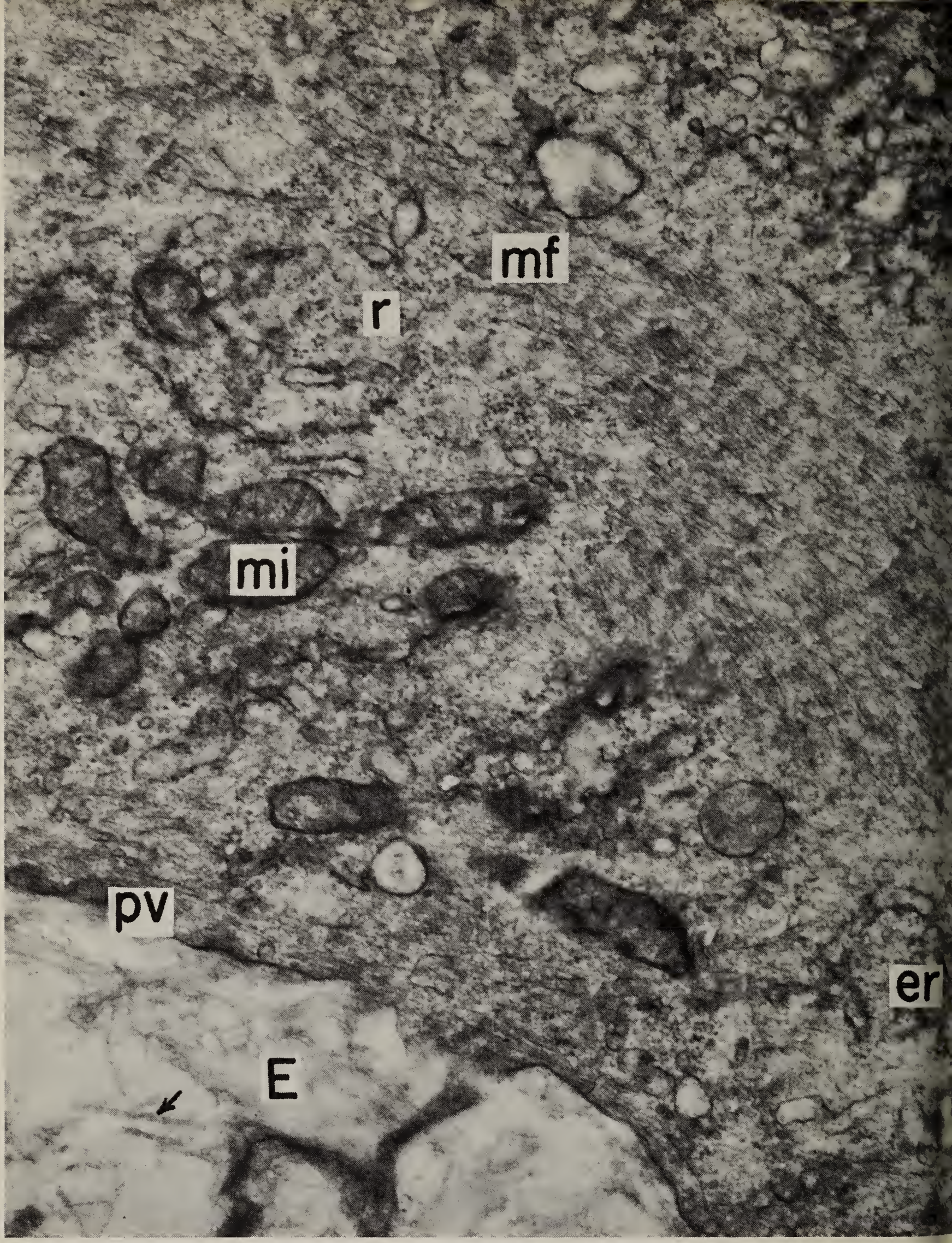

Fig. 2.—Modified smooth muscle cell from intimal plaque covering a lesion similar to that in Fig. 1. The presence of myofilaments (mf) indicates that this is a smooth muscle cell. Other organelles include mitochondria (mi), vesicles of pinocytosis (pv), smooth surfaced endoplasmic reticulum (er) and ribosomes (r). Extracellularly, branching elastic fibers (E) and unit fibers of collagen (arrow) are evident.

of the binding appears to be that of ion exchange, since calcium can displace sodium from this tissue. There can be no doubt that the elastic fiber itself calcifies, for electron micrographs of calcified rabbit arteries (Fig. 1) show large numbers of small, needle-like crystallites, almost certainly apatite, within elastic fibers. The fact that crystals are smaller within elastic fibers than in surrounding tissue is further evidence that this protein influences the process of crystallization.

This is of special interest in relation to atherosclerosis, since there is good evidence that arterial medial degenerative disease, particularly calcification, predisposes to atherosclerosis. The cells which proliferate in an arterial wall in response to arterial calcification are mostly modified smooth muscle cells quite similar to those found in other types of arterial injury (Fig. 2).

Further investigations along this line will be concerned with the site of binding of calcium to elastin, the changes in the fiber which cause the increased calcium affinity and ultimately, it is hoped, the causes of this change.

There has been no attempt in this discussion to review the immense literature on calcification. Rather, this has been a discussion of the direction of our own research.

SUMMARY

In the abnormal calcification due to hypervitaminosis D the chemical sequence of events in renal calcification includes: hypercalcemia, an increase in the amount of calcium in the kidney and deposition of calcium phosphate in organic matrices, some of which are newly elaborated and contain acid mucopolysaccharides.

Calcification occurs within elastic tissue in arterial walls. In human aging, this appears to be related to a change in the composition of elastic tissue by which its capacity to bind calcium is increased. The precise nature of this change is as yet unknown.

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Thanks are due to several student research fellows who have contributed to these investigations, and to Hellen Ellis and Leonas Zeruolis for their excellent technical help, and to Dr. George M. Hass for his advice and support.

QUANTIFICATION OF CLOSED CHEST CARDIAC MASSAGE

JAY SILVERMAN, M.D.
JOHN S. GRAETTINGER, M.D.

Following the reintroduction of the simple but monumental concepts of mouth-to-mouth breathing in 1954[1], and closed chest cardiac massage in 1960[2], these techniques have proven themselves in many hands, and are now generally accepted principles in the management of the patient with cardiac arrest. The purpose of this report is to document a case of ventricular fibrillation in which these techniques were effectively employed, and in which intra-arterial blood pressure and the electrocardiogram were monitored before, during, and after the arrest.

The patient was a 49-year-old white male who had had acute rheumatic fever at age 15. He had been rejected by the military because of heart disease, but remained asymptomatic until age 46, at which time he noted the gradual onset of intermittent right upper quadrant distress, palpitation, and easy fatigability. He felt quite well otherwise, and continued at his job as a millwright.

Physical Examination: On examination the pulse was 56 and irregular. The blood pressure was 120/50. The point of maximal impulse was in the sixth interspace at the anterior axillary line and was heaving. Left ventricular diastolic rippling was noted. A systolic thrill was present at the apex. There was no precordial bulge, parasternal lift, or increased retrosternal dullness. The first heart sound was markedly decreased at the apex. S_2 was normally split at the base, with slight accentuation of the pulmonic closure. A third heart sound was present at the apex. Grade IV/VI pansystolic and grade II/VI mid-diastolic murmurs were heard at the apex. A grade II/VI systolic ejection murmur was heard in the second right interspace, and a grade II decrescendo diastolic blow was audible along the mid-left sternal border. The remainder of the physical examination was within normal limits.

Laboratory Findings: The electrocardiogram (Fig. 1) revealed atrial fibrillation with an occasional ectopic ventricular beat, an intraventricular conduction defect, left ventricular hypertrophy, and suggested the possibility of an old anteroseptal myocardial infarction. Chest x-rays (Fig. 2) demonstrated dilatation of the left atrium and both ventricles, predominantly the left. There was no evidence of valvular calcification at fluoroscopy. Right and retrograde left heart catheterizations were performed during rest and exercise without untoward incident (Table I). The salient findings confirmed the clinical impression of severe mitral insufficiency, and moderate aortic insufficiency. The net forward cardiac index was low (2.17 L./min./M^2), and rose only 19% during exercise.

Selective angiocardiography was then attempted from the left ventricle and the aortic root. The aortic pulse pressure tracing and simultaneous electrocardiogram are illustrated in Figure 3. During the retrograde aortic passage, the catheter entered the right coronary artery. Immediately after withdrawal of the cath-

From the Section of Cardiorespiratory Diseases, Department of Medicine.

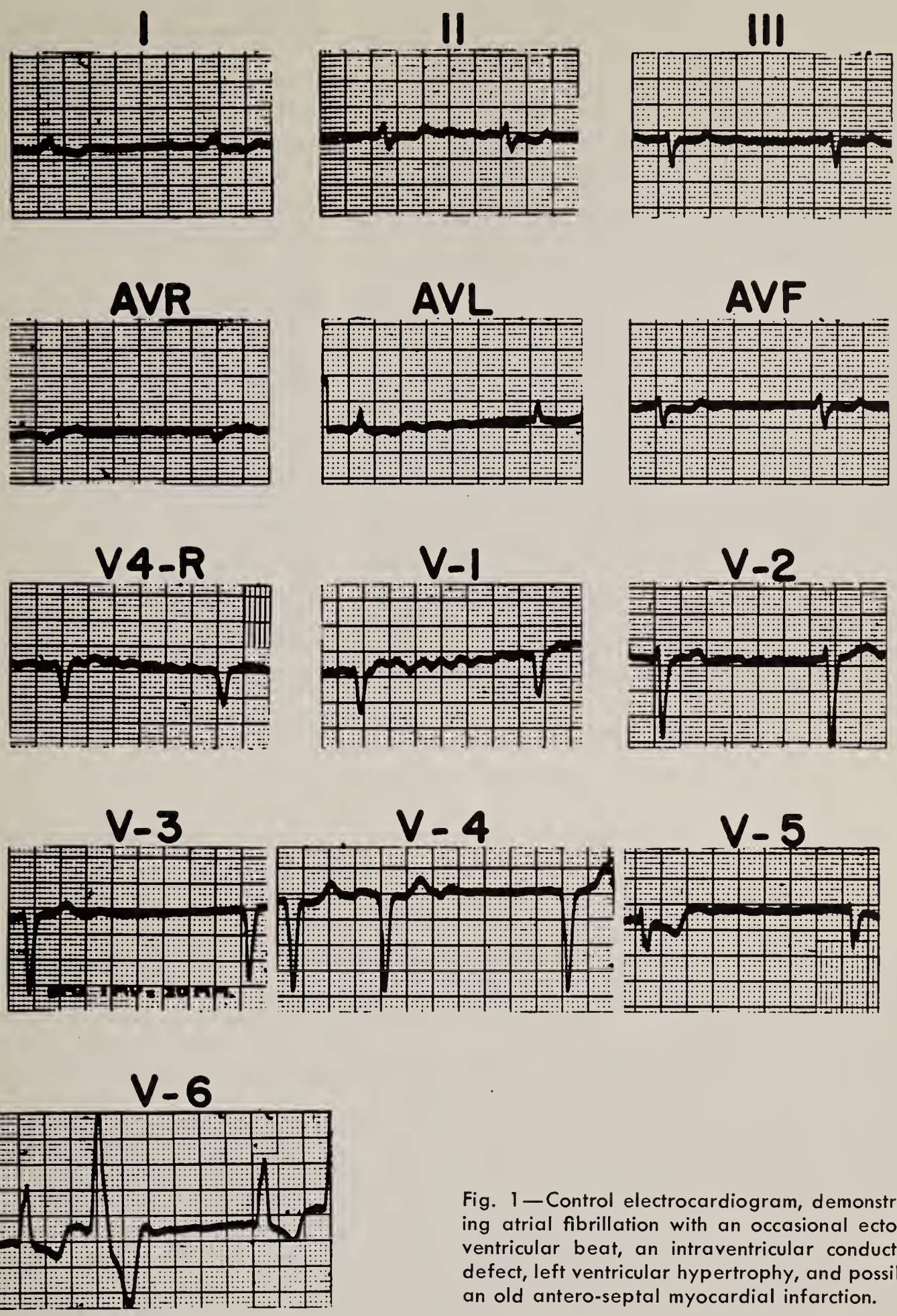

Fig. 1—Control electrocardiogram, demonstrating atrial fibrillation with an occasional ectopic ventricular beat, an intraventricular conduction defect, left ventricular hypertrophy, and possibly an old antero-septal myocardial infarction.

eter tip into the aortic root, the onset of ventricular fibrillation was noted on the monitoring oscilloscope, and the patient lost consciousness. Mouth-to-mouth artificial respiration and closed chest cardiac massage were instituted immediately. A blood pressure which was adequate to maintain coronary and cerebral perfusion (117/52) was maintained (Fig. 4), and the patient rapidly regained consciousness. The arrhythmia persisted. The patient was cooperative, and did not resist the external massage and the passage of an endotrachial tube. He was maintained on 100% oxygen and external cardiac massage for 5½ min-

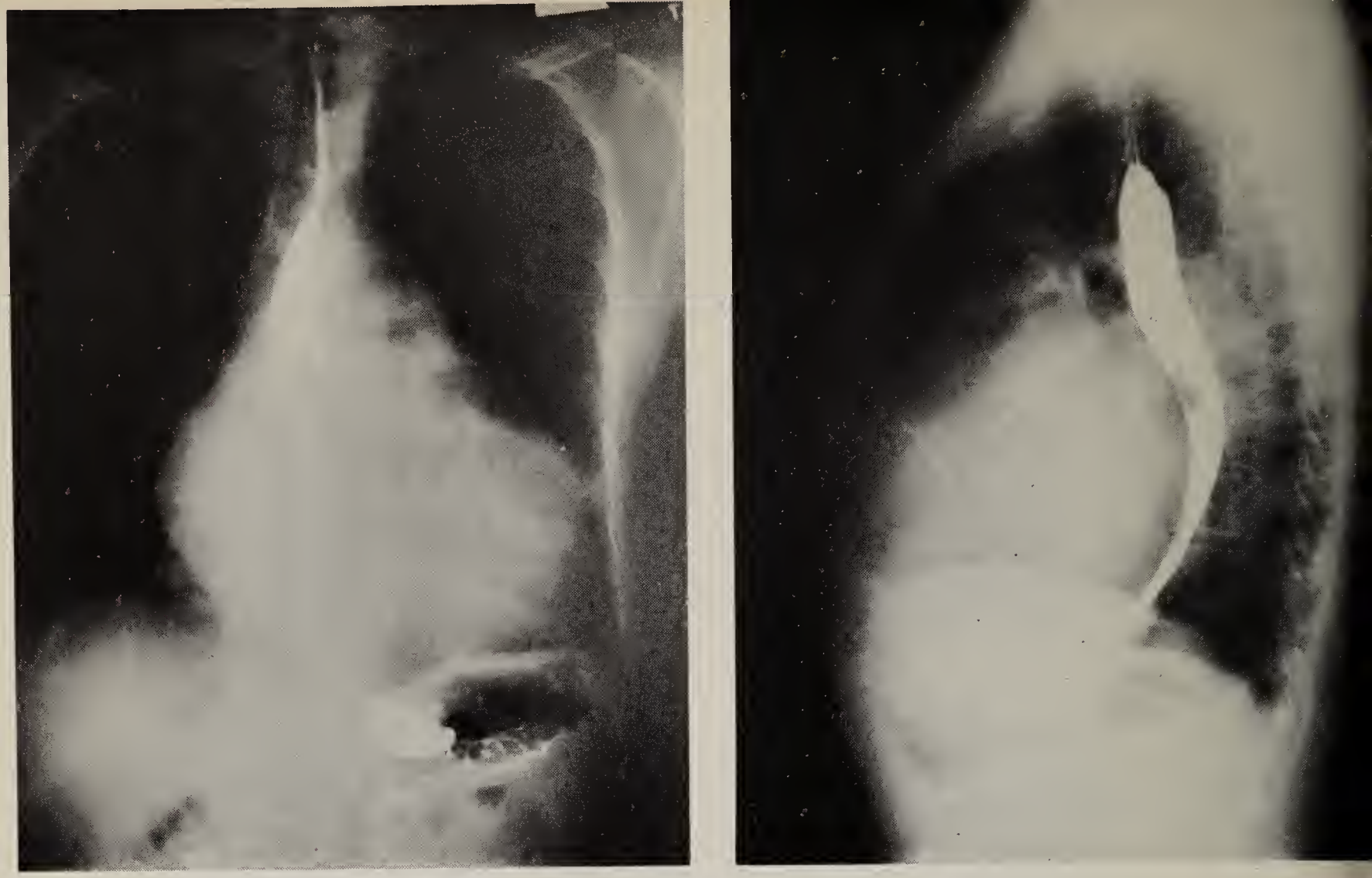

Fig. 2.—Chest roentgenograms with barium swallow, postero-anterior and left lateral views. The left atrium and both ventricles, predominantly the left, are dilated. There is no evidence of valvular calcification.

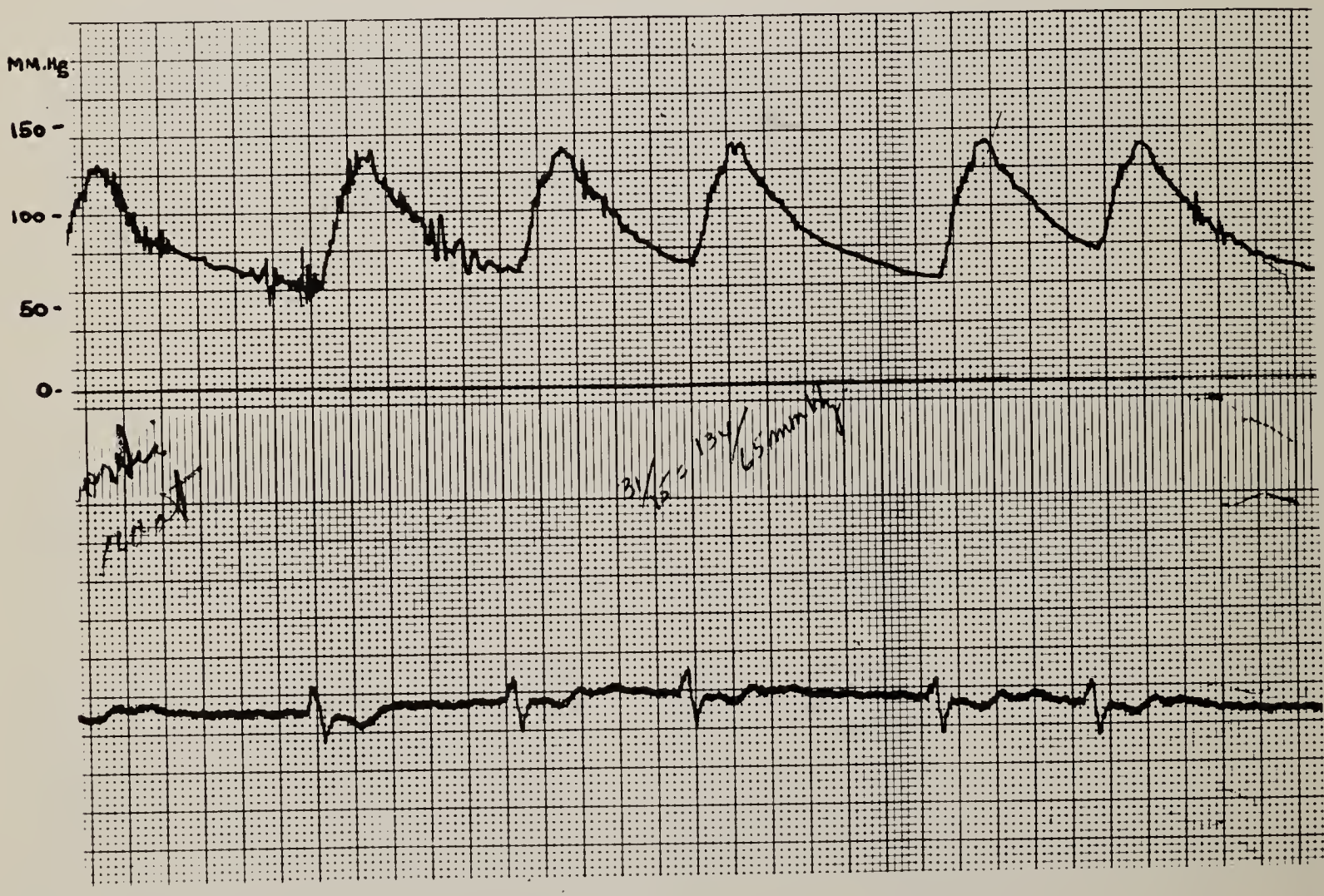

Fig. 3.—Control aortic root pressure pulse (134/65) and electrocardiogram (lead II).

TABLE I: CARDIAC CATHETERIZATION DATA

STATE	HR	$\dot{V}O_{2I}$	C_{AO_2}	PRESSURE, mm. Hg.						CARDIAC INDEX	RESISTANCE (Dynes sec.cm.$^{-5}$)		
				RA v y	RV	PA	"W" v y	LV	AORTA	(L./min./M^2)	TS	TP	PULMONARY ARTERIOLAR
Rest	46	142	94.2%	7 4	36/4	36/15	24 10	11	5/9 127/54	2.17	1570	488	191
Exercise	72	218	93.3%	--	-	60/27	37 11	—	—	2.58	1782	766	410

LEGEND

HR—Heart rate $\dot{V}O_{2I}$—Oxygen consumption index (mls./min./M.^{2}) TP—Total pulmonary
TS—Total systemic C_{AO_2}—arterial oxygen saturation "W"—Wedge

utes, at which time an external countershock of 750 volts was applied for 0.25 seconds. Ventricular fibrillation persisted, and cardiac massage was reinstituted. During the seventh minute of arrhythmia, a second countershock of equal magnitude was applied. This resulted in reversion of the rhythm to atrial fibrillation with a slow ventricular rate. Moderate ST segment elevation was noted in the monitoring lead (AVL) following resumption of the pre-catheterization rhythm (Fig. 5A). A transient episode of bigeminy occurred eight minutes after resumption of atrial fibrillation; the ST segments returned toward normal, and the T waves became diphasic (Fig. 5B). Twenty minutes after resumption of spontaneous rhythm, the ST segment in lead AVL was normal, and the T wave was inverted (Fig. 5C). Two hours later, the electrocardiogram had returned to its pre-operative status.

The electrocardiogram remained stable throughout the remainder of the hospital course. The serum oxaloacetic transaminase level rose to 156 units 24 hours after the catheterization, and returned to the normal range within three days.

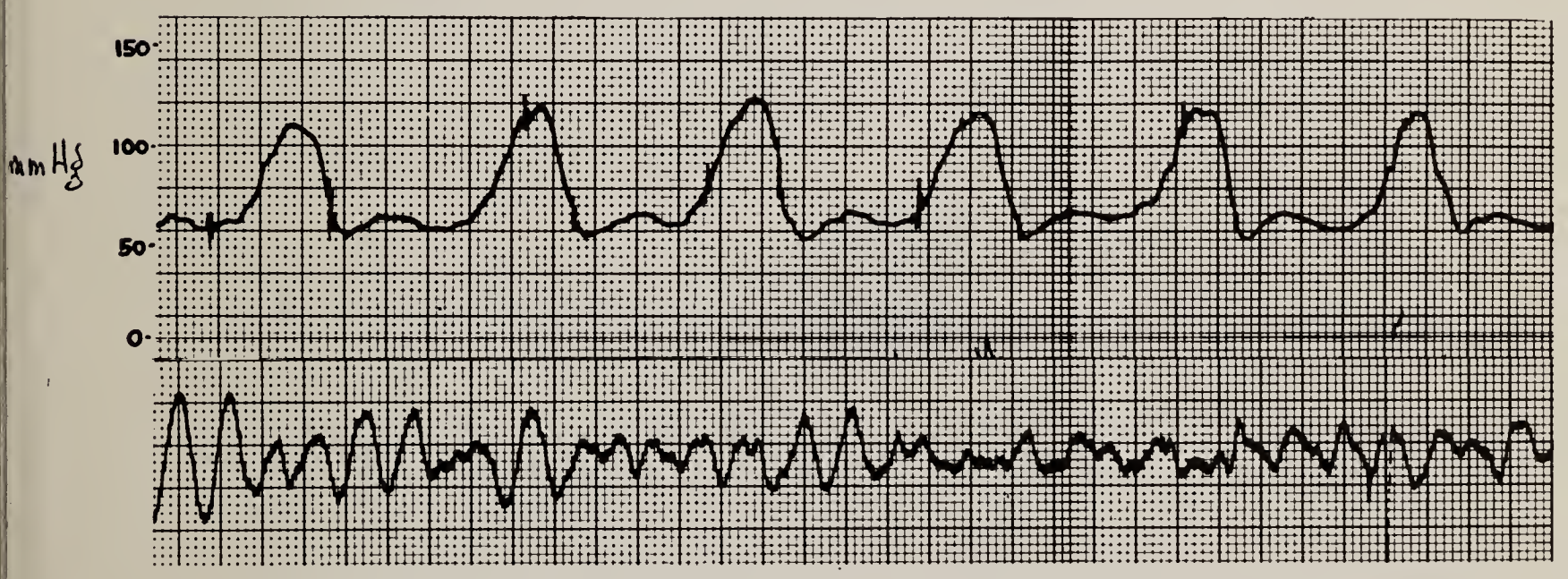

Fig. 4.—Aortic root pressure pulse (117/52) obtained during the external cardiac massage. The electrocardiogram reveals ventricular fibrillation.

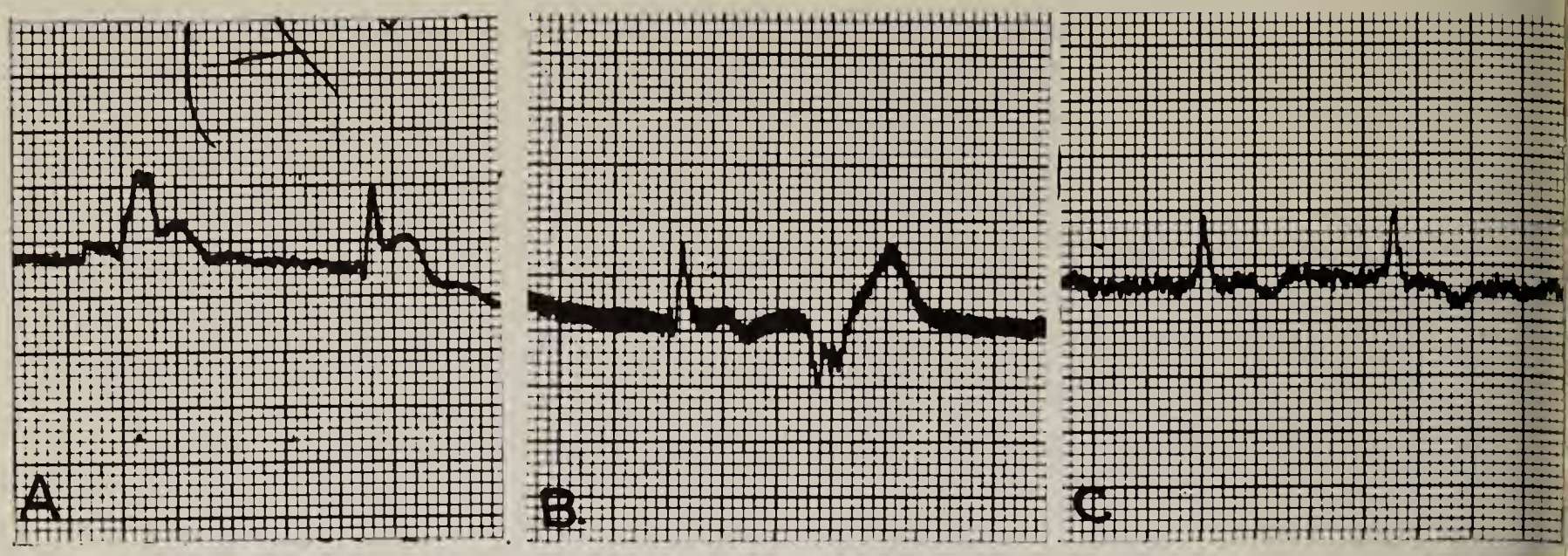

Fig. 5.—Electrocardiogram, lead AVL: (A) Immediately after second countershock and restoration of atrial fibrillation: moderate ST segment elevation is present; (B) Eight minutes after restoration of atrial fibrillation: transient bigenimy, normal ST segment, and diphasic T wave; (C) Twenty minutes after restoration of atrial fibrillation; T waves are inverted.

SUMMARY

An incident of ventricular fibrillation occurring during physiologic studies, and its treatment, are reported. External cardiac massage was instituted immediately; intra-arterial blood pressure was monitored throughout the procedure, and was found to approximate the control blood pressure. The patient was resuscitated without residual cardiovascular or cerebral damage.

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THE TREATMENT OF COMPLETE HEART BLOCK WITH ELECTRONIC PACEMAKERS

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INTRODUCTION

The presence or absence of symptoms in a patient with complete heart block is a highly individualized situation, concerned not only with the basic slow rate of ventricular contraction, but also mutually concerned with the stroke volume of the ventricles, the electrical stability of the myocardium, the coronary blood supply, and other less clearly defined factors. It is common experience to encounter one patient with a ventricular rate of 35 to 40 per minute who is free of symptoms with moderate exercise; a second patient with the same rate who shows signs of congestive heart failure with the slightest exertion; and a third with the same rate who is having bouts of unconsciousness due to periods of cardiac standstill or ventricular tachycardia. Some of these patients are asymptomatic with ventricular rates of 30 per minute, while others have Stokes-Adams attacks with basic rates of 45 per minute, due to the interposition of ventricular arrhythmias. Whatever the symptom complex presenting in the patient, primary treatment is directed to increasing the heart rate.

It is beyond the scope of this communication to discuss at length the physiologic gains accrued by increasing the rate, but it is pertinent at least to mention the increase in cardiac output and coronary blood flow, the reduction in the extremes of stroke volume necessary for output at the slow rates, and the shortening of the prolonged periods of diastole during which the hypoxic myocardium is receptive to aberrant stimuli.

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Many, perhaps the majority, of the symptomatic patients are adequately controlled by stimulation of the heart with sympathomimetic drugs, the most commonly utilized being isuprel, ephedrine, and epinephrine. As an additional aid to medical management, digitalis and diuretics are effective in controlling congestive heart failure, and should be administered when indicated without regard to the slow heart rate. The use of pronestyl and quinidine in the treatment of the associated arrhythmias is poorly tolerated in this group of patients, and is contraindicated.

In a number of patients the heart does not respond to drugs, or, with the passage of time, becomes refractory to the previously effective medication. It is in this group that the substitution of an electronically-induced ventricular stimulus for the intrinsic myocardial pacemaker has developed as a method of restoring adequate cardiac function and stability by activating ventricular contractions at a faster rate. Since May, 1961, electronic pacemakers have been utilized by the authors in the treatment of ten patients with symptoms due to complete heart block. The following discussion is a presentation of this experience, submitted for the purpose of defining the advances and the results which may be achieved with this relatively new form of therapy.

PATIENT SELECTION

The complete heart block in each of the ten patients was presumed to be on the basis of arteriosclerotic heart disease. Four of the ten were known to have had previous myocardial infarctions, and two others described recurrent symptoms characteristic of angina. The block was known to have been present in one patient for three years and in three patients for two years, with symptoms easily controlled by medication until several days or weeks prior to admission. Each of the patients had suffered three or more episodes of syncope, and these episodes constituted the major indication for use of the pacemaker in all but one. The indication for operation in the one exceptional patient was a progressive decrease in heart rate unresponsive to medication, associated with symptoms of low cardiac output. Five of the patients had been digitalized prior to operation for treatment of congestive heart failure. Four were 70 to 74 years of age, five were 66 to 69 years of age, and one was 54 years old. The basic ventricular rates just prior to operation varied from 20 to 45 per minute.

There was no correlation between the severity of symptoms or the frequency of the bouts of syncope and the basic ventricular rates. However, the presence of gross irregularities in the rhythm was associated consistently with the most hazardous clinical course. The three patients exhibiting this evidence of electrical instability could be maintained only with intravenous isuprel during the short time of preparation taken for emergency operations.

MATERIAL AND METHODS

The pacemakers which were utilized were battery-powered transistorized units with two electrodes to be applied directly to the left ventricular myocardium. The first two of the units were commercially manufactured, the remainder were constructed at the Presbyterian-St. Luke's Hospital (author, R. S.). In each case the heart was exposed through a left antero-lateral thoracotomy incision in the fifth intercostal space. In two cases the connecting wires from the myocardial electrodes were brought out through the chest wall for external attachment to the pacemakers. In the remaining cases, the pacemaker, wires, and electrodes were joined together as single self-contained units to be completely implanted in the patient. These latter pacemakers were buried under the pectoral muscles on the left, with the wires passed through the second or third intercostal space to the electrodes on the myocardium.

The pertinent data on all of the patients are summarized in table I. It will be noted that in three cases the initially implanted pacemaker failed and second units were utilized. In one of these three, a third unit was implanted following failure of the second. Three of the four deaths in the series occurred immediately following failure of the electronic units.

CLINICAL EXPERIENCE

The case reports of three patients are worthy of presentation. **Case No. 1:** I.G. was a 73 year old woman having repeated bouts of syncope during the four days prior to admission to the hospital. She

was known to have had a heart block for the previous five months, with symptoms controlled by sublingual tablets of isuprel. Her ventricular rate was 40 per minute on admission. Six days after entering the hospital, an internal pacemaker with a discharge rate of 80 per minute was implanted. Recovery from the operation was rapid and the patient was discharged on the 10th postoperative day, free of symptoms with a heart rate of 80 per minute. She was completely asymptomatic, with full activity consistent with her age, until four months after discharge when she suddenly began having bouts of syncope again. On readmission to the hospital her heart rate was 36 per minute, and there was no evidence of pacemaker activity on the electrocardiogram. She was again taken to the operating room, the inactive pacemaker was removed, and a second unit was implanted. In placing the new electrodes, it was noted that in areas of the myocardium with considerable scarring from the first procedure, the heart did not respond to the second pacemaker. Upon placing the electrodes in a relatively clear site, however, the response was satisfactory with the heart contracting at the new pacemaker rate of 72 per minute. Examination of the unit which was removed revealed a leak in the casing resulting in a short circuit. One of the electrodes was also corroded. Her postoperative course was again satisfactory until the 10th day when she suddenly developed signs of a cerebral injury. On the 21st postoperative day her heart rate, after remaining consistently at 72 per minute, was found to have dropped to 36 per minute. An electrocardiogram showed the pacemaker still discharging at a rate of 72 per minute, but with no response from the ventricles which were contracting independently at the preoperative rate of 36 per minute. Since the patient's cardiac function was satisfactory at the level of inactivity imposed by a hemiplegia, placement of a third pacemaker was considered inadvisable. Following slow partial recovery from the cerebrovascular accident, she was transferred to a nursing home where she is still living with a partial disability. Her heart rate remains at 36 per minute with the pacemaker still in place but not functioning.

Case No. 2: M. B. was a 67 year old man admitted to the hospital as an emergency having incessant repeated bouts of unconsciousness which began that day. The electrocardiogram showed that these episodes were due to periods of cardiac standstill or ventricular tachycardia converting spontaneously to an idioventricular rhythm with a rate of 20 to 30 per minute. With intravenous isuprel the basic rate was increased to 30 to 36 per minute, but the bouts of irregularity and arrest continued. In transit to the operating room, this patient went into ventricular fibrillation. Manual external compression of the heart was carried out until reaching the operating room where the chest was opened, the heart was defibrillated with electric shock, and a commercially constructed pacemaker with a discharge rate of 54 per minute was attached to the myocardium. The heart responded immediately to this pacemaker, but at the still relatively slow rate of 54 per minute, extra-ventricular systoles interceded repeatedly into the long diastolic intervals, and on three consecutive occasions ventricular fibrillation recurred. This pacemaker was detached from the electrode wires and an external pacemaker with a variable rate control was attached. At a pacemaker rate of 88 per minute the extrasystoles were completely obliterated and the patient promptly stabilized. His recovery was prompt and within a week he was fully ambulatory without symptoms. On the ninth postoperative day, however, he suddenly collapsed and could not be resuscitated. At autopsy one of the myocardial electrodes was found to be broken.

Case No. 3: G. E. was a 67 year old man who had been followed for five months with a known previous myocardial infarction and a complete heart block, asymptomatic with a ventricular rate of 45 to 50 per minute. On the day of admission to the hospital he began

TABLE I
DATA ON PACEMAKER IMPLANTATIONS

Patient, Yrs. of Age	Duration of Block	Duration, Kind of Symptoms	Ventricular Pulse Rate	Pace-maker Pulse Rate	Duration of Function, Result	Comment
I.G., 73	*(1) 6 mos.	4 days, syncope	40, reg.	80	4 mos., failure	Casing leak, electrode corrosion.
	(2)	1 day, syncope	36, reg.	72	21 days, failure	Resistance at electrode site heart not responsive.
M.B., 69	Unknown	1 day, syncope	20 to 30, irreg.	88 **(Ext)	9 days, failure	Broken electrode, patient died suddenly.
G.E., 67	5 mos.	1 day, syncope	22, irreg.	80(Ext)	13 mos., excel.	Pacemaker functioning well, patient asymptomatic.
L.M., 69	9 mos.	9 mos., syncope	30, reg.	70	29 days	Died of stroke, pacemaker functioned well until death.
F.N., 54	(1) 2 mos.	2 mos., syncope	22, reg.	72	2 mos., failure	Casing leak, broken wire.
	(2)	syncope	30, reg.	80	21 days, failure	Resistance at electrode site heart not responsive.
	(3)	syncope	30, reg.	80	7 mos., failure	Battery failure, death; pulse rate increased prior to failure
M.B., 66	(1) 3 wks.	3 wks., syncope	45, reg.	78	10 days, failure	Broken wire, death.
	(2)	Heart failure	48, reg.	78	28 days, failure	Resistance at electrode site.
T.A., 69	6 mos.	2 days, syncope	20, irreg.	76	3 mos., excel.	Functioning well, pt. asympt.
D.K., 76	2 yrs.	2 mos., syncope	28, reg.	76	2 mos., excel.	Functioning well, pt. asympt.
M.M., 74	3 yrs.	2 wks., syncope	32, reg.	72	6 wks., excel.	Functioning well, pt. asympt.
O.H., 73	2 yrs.	3 wks., decreasing pulse rate.	40-22, reg.	78	3 wks., excel.	Functioning well, pt. asympt.

*( ): Numbers indicate sequence of pacemakers in patients in whom two or more were implanted.
**(Ext): Electrode wires through chest wall and attached to external pacemaker unit.

having repeated Stokes-Adams attacks. His electrocardiogram showed a basic ventricular rate of 22 per minute with multiple extrasystoles and episodes of cardiac standstill. With intravenous isuprel the heart rate increased to 30 per minute but the episodes of standstill and syncope persisted. On the third hospital day he was taken to the operating room and electrodes were attached to the left ventricular myocardium. Because an implantable pacemaker was not available at that time, the electrode wires were brought out through the chest wall and attached to an external transistorized battery-powered pacemaker. The heart responded promptly to its rate of 80 per minute. His postoperative course was uneventful and he was discharged two weeks later completely free of symptoms. This patient has now been maintained with a pacemaker for 13 months. He is fully active, has returned to work, and has no complaints of physical disability. A small amount of purulent discharge persists at the site of exit of the electrode wires, but the infection is superficial and has caused no difficulties. The pacemaker unit was changed nine months after operation in order to study the extent of alteration of the first unit after a prolonged period of use.

DISCUSSION

The cases presented above are useful for demonstration of the excellent response to specific therapy which may be achieved with these electronic units. In all cases, despite the precarious condition of most of the patients, operation has been well tolerated. The implanted pacemakers have caused no discomfort and there have been no wound infections other than

that described in the third case with the wires traversing the chest wall to an external unit. During the periods of satisfactory function of the pacemakers, the patients have had no episodes of syncope, and all have described significant improvement in exercise tolerance. An interesting phenomenon has been noted in the response of blood pressures to the increase in heart rate (Table II). All of the patients preoperatively showed a widened pulse pressure with elevation of the systolic pressure and a lowered diastolic pressure. With acceleration of the heart rate by the pacemakers, blood pressures returned to normal or near normal levels.

The first two of the cases presented above are of special importance in that they illustrate several of the problems of physiologic application and unit construction which could be appreciated only by experience. The first of the problems encountered, leakage of the casing, occurred in two patients. The resulting short circuit in case no. 1 gave a continuous discharge of current which was responsible for the corrosion of the platinum electrode, but fortunately depleted the batteries and was emitted at an insufficient voltage to stimulate the heart. In case no. 5, the leak in the casing was demonstrated in the laboratory, but it is doubtful that this was responsible for the unit failure.

The failure of two units was attributed to insufficient voltage to overcome resistance at the site of electrode implantation, in hearts which had had a previous set of electrodes implanted. Whether this increased resistance is actually due to scarring at the electrode site or to a decreased receptibility of the myocardium or to an interference with transmission of the stimulus through the scarred myocardium has not been demonstrated. It has been shown, however, that higher voltage levels than those produced by the specific pacemakers in these cases will activate the myocardium despite the scarring.

Breakage of an electrode or a connecting wire occurred in three cases. This has been a major cause of pacemaker failure in all reported series. The wires in each case were of twisted strands of steel, sutured into the myocardium by means of a swedged-on needle. Breakage occurred at the site of maximum flexion where the wires entered the myocardium. This type of wire is no longer being utilized, either by us or by most other workers in this field. The wires now in use will be described below.

Battery failure occurred in one instrument, and this is a complication which can probably never be entirely eliminated. Decay in the batteries, however, can be recognized well before complete failure occurs by changes in the pulse rate. In this patient the unit was constructed to increase the pulse rate as battery decay occurred. On an outpatient visit three weeks before complete

TABLE II
BLOOD PRESSURE RESPONSE TO CHANGE IN PULSE RATE

Patient	Preoperative		Postoperative	
	Pulse Rate	B. P.	Pulse Rate	B. P.
I. G.	(1) 40	214/110	80	150/100
	(2) 36	230/100	72	130/70
L. M.	30	210/80	70	110/80
F. N.	(1) 22	190/80	72	120/80
	(2) 30	204/140	80	120/80
M. B.	45	200/60	78	120/80
D. K.	28	160/50	76	130/72
M. M.	32	178/80	72	130/82
O. H.	40	176/70	78	130/80

failure of the unit, a significant rate increase was recorded on the electrocardiogram but was not appreciated by the examining physician. This rate increase should have signaled the necessity for an immediate unit change. The pacemakers now in use are constructed to show a decrease in rate with battery decay. The patients are all instructed to count their pulse at least once daily, and they as well as their family and physicians are instructed in regard to the significance of the change in rate. It should be added that changing of a pacemaker under these circumstances is a relatively simple procedure, requiring only removal of the electronic unit from the sub-pectoral pocket and attachment of the new unit to the original wires. The thoracic cavity is not entered and the myocardial electrodes are not disturbed.

Determination of the most desirable rate at which these pacemakers should be set has caused some controversy and much concern. In case no. 2, the internal pacemaker secured from a commercial firm was set at a rate of 54 per minute, as were all of the pacemakers manufactured by that firm. This relatively slow setting was based upon experimental work by their medical consultants showing that ventricular stroke volume was at a maximum level at rates of 50 to 60 per minute, and that a rate increase reduced stroke volume while not appreciably increasing the total cardiac output. Since these determinations were made at rest, however, utilization of the maximum stroke volume with a fixed rate allows no increase of output during exercise. It is therefore probably desirable to attain a satisfactory resting cardiac output by means of a somewhat faster rate so that a reserve in stroke volume may be utilized with exercise.

A second, and equally important, disadvantage of pacemaker rates in the range of 50 to 60 per minute was well demonstrated in case no. 2, in which the relatively long periods of diastole afforded at a rate of 54 per minute allowed the intervention of stimuli from ventricular foci. This phenomenon has been demonstrated in our laboratories by Dr. Takashi Iwa. Experimentally and clinically, pacemaker-induced rates of 70 to 80 per minute have sufficiently shortened the diastolic interval that idioventricular impulses have been eliminated, and it is our opinion that slower rates should not be utilized.

The pacemakers now being used by our group have been designed, after repeated alterations, to eliminate insofar as possible the deficiencies of the earlier units. These latest models, implanted in the last four patients in the series, are 6x8x2 centimeters in external dimensions and weigh 175 grams. The electrical components consist of two transistors, one for blocking of the oscillator and one for constant current output; one transformer; three capacitors; three resistors; and nine mercury batteries, three of which are utilized to power the circuit. The total battery rating available for stimulation of the heart is 8.5 volts for 1000 milliampere hours. The units are constructed to deliver monophasic impulses at a fixed rate of 70 to 80 per minute, two milliseconds in duration, 8.5 volts in intensity, and approximately 15 milliamperes in current across the electrodes with a resistance of 800 ohms. The approximate time that current is discharged is 15 hours per year. After assembly, with the electrode wires attached, the components are imbedded in an epoxy resin, completely sealing all parts. The entire unit including the wires but with the exception of the electrodes, is then enclosed in a smoothly finished case of silastic. (Figs. 1 & 2)

The wires consist of four strands of flexible, fatigue-resistant steel coiled as a helical spring so that flexion is distributed over a considerable length of each strand. The electrodes are discs of platinum nine millimeters in diameter, with a backing of epoxy resin through which two holes are provided for suturing to the myocardium. Maximum efficiency of performance is attained by placing these discs on the myocardium one centimeter apart.

Fig. 1

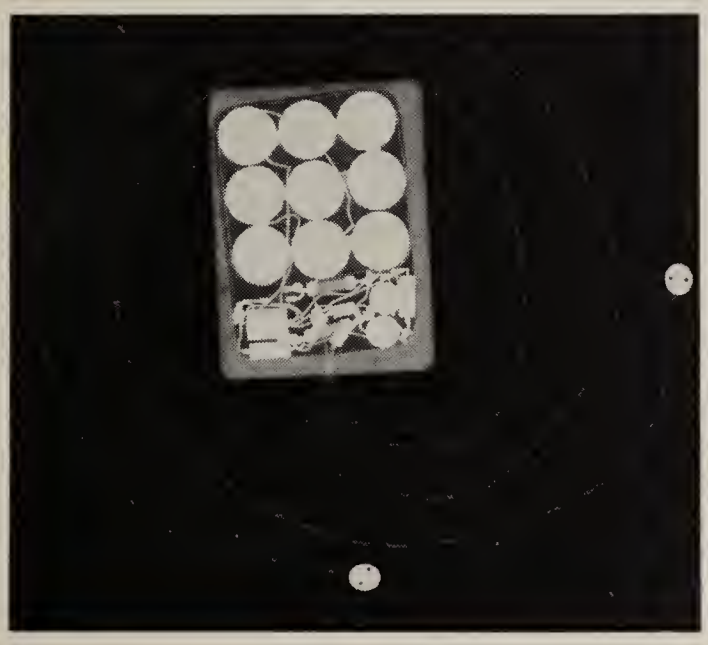

Fig. 2

It is our opinion that these electronic pacemakers now provide a sound and effective method of treatment in those patients with complete heart block whose symptoms are not controlled by medical management. This group of patients should include those with cerebral symptoms due to ventricular standstill or tachycardia, as well as those whose symptoms are due to low cardiac output or congestive heart failure. Although a clear relationship between the ventricular rate and mortality or morbidity has not been documented, the pacemakers should probably be implanted in all patients with heart rates of 30 per minute or less, even in the absence of symptoms. Their use should also be strongly considered in those patients showing persistent irregularities of rhythm in association with complete heart block, even in the absence of symptoms.

SUMMARY

Ten patients with symptomatic complete heart block have been treated by implantation of myocardial electrodes connected to electronic pacemakers for stimulation of the heart at rates of 70 to 80 per minute. The increase in heart rate achieved by the use of the pacemakers has effectively controlled Stokes-Adams attacks or symptoms of low cardiac output in all patients. The causes for failure of the pacemakers after varying periods of time in four patients include leakage of the pacemaker casings, breakage of the electrode wires, increasing resistance to stimulation at the electrode site, and battery failure. It appears promising that the deficiencies leading to failure in the early cases have been corrected in the units now in use, and that implantation of these pacemakers is a sound and effective method of treating this group of severely ill patients.

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THE CLINICAL SIGNIFICANCE OF VARIATIONS IN THE URINARY EXCRETION OF AMINO ACIDS

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Amino acids are normally absorbed by the small intestine, carried in the plasma, filtered by the renal glomerulus, and reabsorbed by the kidney tubules. Their ultimate destination is, of course, incorporation into body protein. However, about 10 per cent of the total filtered amino acids are normally excreted in the urine. Those which are found most often in urine from normal adults are glycine, serine, taurine, alanine, histidine, the methyl histidines, lysine, and beta-aminoisobutyric acid. Physiologic variations in normal persons are shown in table I.

TABLE I
PHYSIOLOGIC PATTERNS OF URINARY AMINO ACIDS

Condition	Pattern of Excretion
Normal adults, infants and children	Glycine with or without glutamine, alanine, serine, taurine, histidine, methylhistidine, beta-aminoisobutyric acid, lysine.
High protein diet	Normal plus increased taurine, histidine, and methylhistidines.
Pregnancy	Normal plus increased histidine, lysine, phenylalanine, tyrosine
Infants (new born-full term)	Normal plus increased taurine (7 days) and occasionally cystine, proline, hydroxyproline, tyrosine, leucine, valine, methionine, glutamic acid.
Premature infants	Generalized aminoaciduria (physiological)

The advent of the relatively simple technique of paper chromatography has permitted the screening of large numbers of urine samples. As a result, to date, over forty distinct clinical syndromes with associated aminoaciduria have been reported (see table II for list of causes of aminoaciduria). Interest in clinical implications of aminoaciduria was stimulated by C.E. Dent[1,2] and has now become universal[2-9]. Although aminoaciduria is often associated with rare diseases, it is of importance for at least three reasons: (1) it is associated in some instances with treatable disease, such as galactosemia, vitamin D deficiency rickets, phenylketonuria, cystinuria, lead poisoning, and the congenital form of renal tubular acidosis; (2) study of disturbances of amino acid metabolism and excretion may give useful information regarding normal metabolic pathways and transport systems, e.g. as in cystinuria and tyrosinosis; and (3) study of urinary amino acids is useful in surveying asymptomatic relatives of a

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This work was done during tenure of a postdoctoral fellowship, N.H.I., U.S.P.H.S.

given patient and, thus, aids in the elucidation of genetic mechanisms as well as leading to early diagnosis in affected individuals.

Disturbances in amino acid metabolism and urinary amino acid excretion are either inherited or acquired and may be manifested as either a generalized aminoaciduria (Wilson's disease, Fanconi syndrome, vitamin D deficiency) or a more specific abnormality (cystinuria, phenylketonuria and "maple syrup urine" disease).

In general, there are 3 types of abnormal aminoaciduria. The first is "overflow aminoaciduria" where the abnormal excretion is caused by elevated levels of plasma amino acids. Examples of this are phenylketonuria, acute yellow atrophy, and maple syrup urine disease.

The second type is "no threshold aminoaciduria," in which there is normally none of the particular amino acid present in the plasma. Strictly speaking, this is also a form of overflow aminoaciduria. Phosphoethanolaminuria in hypophosphatasia, the inherited beta-aminoisobutyricaciduria of normal persons, and arginino-succinic-aciduria are examples of this form.

The third type is "renal aminoaciduria" in which the normal reabsorptive mechanisms of the renal tubules are at fault. The aminoaciduria of vitamin D deficient rickets, galactosemia, Wilson's disease, the nephrotic syndrome, scurvy and cystinuria are of the "renal type."

Also, there is a difference between "hepatic" and "renal" patterns of aminoaciduria. In general, severe liver disease is charcterized by the prominent excretion of sulfur containing amino acids (taurine, cystine and methionine), ethanolamine and beta-aminoiso-butyric acid[2,7,8].

SPECIFIC DISTURBANCES IN AMINO ACID METABOLISM, TRANSPORT AND EXCRETION

The scope of this presentation does not permit discussion of all the various causes of aminoaciduria and several reviews are

TABLE II
Conditions Associated with Aminoaciduria
(Modified from Andrews[3] and Rowley et. al [13])

- **I. Overflow type**
  - A. Acquired
    1. Severe liver damage
  - B. Inherited
    1. Phenylketonuria
    2. Maple syrup urine disease
    3. Cystathioninuria
    4. Histidinemia (10,11)
    5. Citrullinuria (15)
    6. Idiopathic hyperglycinemia (17)
    7. Inborn error of tyrosine metabolism with convulsions and failure to thrive (12)
- **II. "No Threshold" type**
  1. Beta-amino-isobutyric-aciduria
  2. Argininosuccinicaciduria
  3. Hypophosphatasia
- **III. Renal type**
  - A. Acquired
    1. Vitamin deficiencies
       a. Scurvy
       b. Vitamin D deficient rickets
       c. Vitamin E deficiency (rabbits)
    2. Potassium deficiency
    3. Kawashiorkor
    4. Poisons (cadmium, uranium, lead, mercury, benzene, salicylates, gasoline, nitrobenzene, lysol)
    5. Maleic and trans-aconitic acids (in ricketic rats)
    6. Acute tubular necrosis
    7. Nephrotic syndrome
    8. Multiple myeloma with adult Fanconi syndrome
  - B. Inherited
    1. Cystinuria
    2. Cystinosis
    3. Adult Fanconi syndrome
    4. Luder Sheldon syndrome (aminoaciduria and glycosuria)
    5. Congenital renal tubular acidosis
    6. Lowe's syndrome
    7. Hartnup disease
    8. Sex-linked microcephaly with diplegia
    9. Convulsive disorder with prolinuria
    10. Baber's congenital cirrhosis
    11. Wilson's disease
    12. Galactosemia
    13. Familial growth retardation and cor pulmonale (13)
    14. Glycinuria with nephrolithiasis
    15. Grand mal epilepsy (22)
    16. Congenital cataracts
    17. Glycinuria and glycosuria (46)
- **IV. Mixed type**
  1. Pregnancy
  2. ? Familial hyperprolinemia (16)
- **V. Unclassified**
  1. Oasthonse disease
  2. Familial glomerulonephritis with aminoaciduria (14)
  3. Petit mal epilepsy (21)

available for the interested reader[2-9]. However, some recently reported syndromes will be mentioned as will some particular relationships of amino acids to other aspects of metabolism.

Syndromes Mimicking Phenylketonuria

Two new disturbances in amino acid metabolism which gaive false positive urine tests for phenylpyruvic acid with ferric chloride and Phenistix® have been described. The first of these is histidinemia[10,11] in which there is speech retardation but normal intelligence, an increase in plasma and urinary histidine, an abnormal excretion of imidazole-pyruvic, -lactic, and -acetic acids, and a deficiency in histidase, the enzyme which is responsible for the conversion of histidine to its metabolic product, urocanic acid.

The second disturbance was reported in an infant with seizures, spasticity, failure to gain weight, hypocalcemia which responded to vitamin D, metabolic acidosis, and abnormal urinary excretion of tyrosine, para-hydroxy-phenylpyruvic, -lactic, and -acetic acids. The patient responded to a low phenylalanine diet with clinical improvement and disappearance of abnormal urinary metabolites. Because of no demonstrable homogentistic acid in the urine, the authors postulated a block in the oxidation of para-hydroxy-phenylpyruvic acid to homogentisic acid[12].

Familial Growth Retardation with Cor Pulmonale

Three of six children of asymptomatic parents were affected by a syndrome characterized by growth retardation, poor muscular development, scanty adipose tissue, recurrent pulmonary infections, atelectasis, and right ventricular hypertrophy. The one surviving patient was also found to have a marked generalized renal aminoaciduria, as were several asymptomatic relatives. The etiology of the syndrome could not be ascertained[13].

Familial Glomerulonephritis and Aminoaciduria

Three of six siblings of unrelated parents died of glomerulonephritis and a fourth of unknown causes in infancy. The surviving two siblings and parents were normal clinically and had normal urinalyses, but also had increased levels of serum alkaline phosphatase and a generalized aminoaciduria. No plasma amino acid studies were reported[14].

Citrullinuria

Overflow citrullinuria was reported in an eighteen month old retarded infant. The urine, plasma, and cerebrospinal fluid all contained markedly elevated levels of citrulline[15].

Familial Hyperprolinemia

A family with nephritis, nerve deafness, "renal hypoplasia," and photogenic epilepsy was found to have genetically independent hyperprolinemia, and aminoaciduria (glycine, proline, and hydroxyproline). The authors postulated a new renal transport system involving the amino acid glycine and the amino acids proline and hydroxyproline on the basis of loading studies[16,45].

Idiopathic Hyperglycinemia and Glycinuria

This syndrome occurred in a newborn infant with normal parents and was characterized by neutropenia, thrombocytopenia, hypogammaglobulinemia, metabolic acidosis, ketonuria, vomiting, lethargy, and developmental retardation. During the first three years of life these episodes were repeated and often preceded by high protein feedings. The patient also had an abnormal EEG and osteoporosis. The plasma and urine glycine were markedly increased. Leucine, isoleucine and threonine administration produced severe clinical symptoms, but no hypoglycemia ensued. No definite block in the metabolism of glycine was demonstrated[17].

Isolated glycinuria has also been reported previously in a hereditary dis-

turbance associated with nephrolithiasis[18], in type I rickets of Dent[19], and in convulsions associated with cerebral atrophy[3].

Aminoaciduria in Neurologic and Muscular Disorders

Pathologic aminoaciduria has been reported in a variety of neurologic diseases, especially those associated with mental retardation[20]. Recently, aminoaciduria involving leucine, isoleucine, cystine, histidine, tryptophan, aspartic acid, glutamic acid, and lysine has been reported in 10 children with petit mal epilepsy[21]. Transient renal aminoaciduria in grand mal epilepsy had been observed earlier[22]. Also, renal aminoaciduria involving carnosine, anserine, histidine, and 1-methyl histidine has been found in five patients from three families with cerebromacular degeneration[23].

Abnormal urinary amino acid excretion in experimental vitamin E-deficient progressive muscular dystrophy is well established but has not been conclusively documented in the human disease. Normal, decreased and increased excretions of urinary amino acids have been reported[24,25,8]. In this institution preliminary studies of urine amino acids in progressive muscular dystrophy have shown no consistent abnormalities of urinary amino acid excretion. Prolinuria has been reported in two patients with progressive muscular atrophy[25] but this remains to be confirmed; it has also been reported in a familial convulsive disorder[26].

Vitamin D and Aminoaciduria

Vitamin D deficiency is characteristically associated with a renal aminoaciduria, and recent studies in Vitamin D-deprived premature infants showed that this abnormality was much more consistent than any change in blood calcium, phosphorus, alkaline phosphatase, or radiographic evidence of rickets[27]. Experimental vitamin D deficiency in rats, however, shows no associated aminoaciduria[28]. An isolated case of aminoaciduria due to vitamin D sensitivity was reported by Van Creveld[29].

Aminoaciduria in Hyperparathyroidism

Aminoaciduria does not ordinarily occur in hyperparathyroidism; however, one case of generalized aminoaciduria associated with a functioning parathyroid carcinoma has been reported[30]. Interestingly, the urinary amino acid excretion returned to normal following removal of the tumor. Renal aminoaciduria in a patient with primary hyperparathyroidism and renal failure was reported by Cusworth and Dent[31].

Nephrolithiasis and Urinary Amino Acids

Most patients with nephrolithiasis have no consistent abnormalities in urinary amino acid excretion[32,33]; cystinuria and the hereditary glycinuria with associated calcium oxalate stones are the only two known instances of aminoaciduria associated with nephrolithiasis. Cystinuria accounts for about one per cent of all urinary stones[6] and only one report of hereditary glycinuria with nephrolithiasis has appeared[18]. Cystinuria also occurs in the Kenya genet, dogs and mink.

Poisoning and Aminoaciduria

Aminoaciduria associated with toxicity of lead, uranium, cadmium, lysol, and more recently with gasoline and salicylates has been reported.[3] The reported study of aminoaciduria associated with salicylate intoxication indicated that the generalized aminoaciduria regressed with treatment[3]. Experimental aminoaciduria has also been produced with maleic and trans-aconitic acid in ricketic rats[3,34].

Urinary Hydroxyproline and Collagen Metabolism

There are two nitrogenous compounds whose distribution is virtually limited to collagen. These are the amino acid hydroxylysine and the imino acid hydroxyproline[35]. An attempt has been made by several investigators to detect changes in collagen metabolism by measuring changes in urinary hydroxyproline (which is almost entirely excreted in the bound

("peptide") form), especially since the development of a specific chemical method for its determination[36]. Children excrete two to three times more total urine hydroxyproline than adults. Studies of total urinary hydroxyproline excretion on gelatin-free diets in patients with rheumatoid arthritis and disseminated lupus erythematosis have so far shown no definite abnormality[37], although isolation of some interesting hydroxyproline containing peptides from urine of rheumatoid arthritis patients has been reported[38,39]. Increased urinary total hydroxyproline has been reported in Marfan's syndrome and the relationship between growth hormone and urinary hydroxyproline has received considerable attention. Available data, in part, support the notion that urinary total hydroxyproline is a reliable index of growth hormone activity and collagen turnover (see table III). Also of interest is the finding of abnormal proline tolerance in patients with osteogenesis imperfecta[40].

The Metabolic Defect in Hartnup Disease

Hartnup disease, originally described by Baron, Dent, Harris, Hart and Jepson is a hereditary disturbance characterized by an intermittent pellagra-like skin rash, cerebellar ataxia, occasional mental aberrations, and a characteristic renal aminoaciduria without any other abnormality in renal function[6]. The most prominent features of the aminoaciduria are absence of any significant abnormality in glycine or proline excretion. Also tryptophan absorption by the jejunum is delayed and increased amounts of indolylactic acid and other tryptophan metabolites are excreted in the urine. Also of significance is the fact that this is the first instance in which an abnormality of cellular transport of amino acids has been demonstrated in tissues other than the kidney in hereditary disease in man[44].

TABLE III

Total Urinary Hydroxyproline in Man

(mg/24 hour on gelatin-free diet)

Author	Ziff et al. (37)	Prockop and Sjoerdsma (41)	Jasin et al. (42)	Dull and Henneman (43)
Normal children	38.4-126.3 mg.	—	70.1 mg. (mean)	27-96 mg.
Normal adults	14.9-33 mg.	16-34 mg.	37.9 mg. (mean)	8-31 mg.
Normal adults plus gelatin	134 mg.	356-427 mg.	—	—
Rheumatoid Arthritis	13.0-62.3 mg.	—	—	—
"Collagen Diseases"	12.0-40.1 mg.	—	—	—
"Miscellaneous Diseases"	11.3-37.1 mg.	—	—	—
Marfan's Syndrome	—	38-91 mg.	—	—
Thyrotoxicosis	—	—	—	79-205 mg.
Primary hyperparathyroidism	—	—	—	99 mg.
Hypoparathyroidism	—	—	—	14 mg.
Hypoparathyroidism plus 6 days parathyroid extract	—	—	—	55 mg.
Paget's Disease of Bone	—	—	—	67-570 mg.
Pituitary Dwarfism (8 yrs. of age)	—	—	14.1 mg. (mean)	34-45 mg.
Pituitary Dwarfism plus human growth hormone	—	—	60.4 mg. (mean)	72 mg.
Acromegaly	—	—	101.5 mg. (mean)	—
Acromegaly (3 mos. post irradiation)	—	—	58.3 mg. (mean)	—

Changes in Amino Acid Patterns During Treatment of Clinical Disease

Attention should be drawn to the fact that in certain amino acid disorders changes occur in excretion of amino acids with treatment. For example, the renal aminoaciduria of galactosemia and vitamin D deficiency disappear following treatment and the plasma phenylalanine sometimes falls with a low phenylalanine diet in phenylketonuria. These changes may be of some help to clinician in following and evaluating treatment.

SUMMARY

Normally, less than ten per cent of the total amino acids in the glomerular filtrate appear in the urine. Abnormal amounts and patterns of urinary amino acids occur in a variety of acquired and hereditary metabolic disturbances and may often be associated with neurologic and mental disease, renal tubular disease, rickets or osteomalacia, or toxicity of a variety of chemicals. Certain of the diseases associated with aminoaciduria are potentially treatable (e.g. galactosemia, Wilson's disease, vitamin D deficiency, and phenylketonuria); others are rare, often hereditary diseases whose study helps provide information regarding biochemical pathways and genetic mechanisms in man.

Determination of urinary amino acids is a useful tool in differential diagnosis of a variety of diseases whose features may be non-specific but which present clinically as muscle, skeletal, renal, or cerebral disturbances. Also, useful information may be made available regarding patterns of inheritance by studying amino acid excretion in the relatives of a given patient.

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CLINICAL APPLICATIONS OF CHROMOSOME ANALYSIS

Rute Medenis, M.D.

In the late 19th century it became clear that each species has a constant number of chromosomes and that this number was constant within the species. Von Winiwarter[13] in 1912 claimed that there were 47 chromosomes in man. Painter[6] in 1923 reported that both males and females have 48 chromosomes with the sex chromosome complement being XY in the male and XX in the female. For almost half a century this conclusion remained unchallenged. In 1956 Tjio and Levan announced that the correct diploid chromosome number in humans was 46. This finding was confirmed subsequently by English and American investigators.

Since the introduction of improved cytologic techniques which made possible a more reliable determination of chromosome number and morphology it became evident that many types of deviations from the normal chromosome pattern were compatible with life and that some abnormal chromosome complements were associated with more or less well defined clinical entities.

The great advances made within the past few years in the field of human cytogenetics are not only of academic interest but have proved to be of interest and importance to the clinician in establishing an accurate diagnosis in some cases.

At present there are three general methods of tissue culture for chromosome analysis in use. Bone marrow aspirates and peripheral blood leukocytes are utilized for short-term cultures. Skin and other tissue biopsy specimens require a long-term incubation. The three methods are regarded at present by most investigators as complementary rather than alternative. The in vitro dividing cells are exposed to minute amounts of colchicine for a short period of time to arrest mitosis in metaphase, contract the chromosomes and disperse them in cytoplasm. Treatment of cells arrested in metaphase with hypotonic solution is used to expand the cell and separate the chromosomes by reducing the viscosity of cytoplasm. Air-drying on a glass surface or squashing the cells between slide and cover-slip promotes further spreading of the chromosomes in a single optical plane. The preparations are stained with DNA stains. Despite apparent simplicity all methods of chromosome preparations have many pitfalls and on some occasions adequate material for analysis is difficult to obtain.

In order to identify human chromosomes several different systems of nomenclature have been used. This resulted in confusion in the literature. In 1960 in Denver, Colorado, a conference of cytogeneticists accepted the Denver International System[14] for classification of human chromosomes. By the Denver system the pairs of chromosomes are classified by their relative length and by the position of the centromere. The first group consists of three pairs of large chromosomes with medium centromere. The group 4-5 are large chromosomes with submedian centromeres. The 6-12 group are medium-sized chromosomes with submedian centromeres. The X-chromosome resembles chromosome 6 from which it is distinguished with diffi-

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culty. This large group presents major problems in the identification of the individual chromosome pairs. Group 13-15 are medium-sized chromosomes with nearly terminal centromeres. They are also called acrocentric chromosomes and they carry satellites on their short arms. The 16-18 group consists of rather short chromosomes with median or submedian centromeres. Chromosomes 19-20 are short with centromeres located in the median position. The group 21-22 are very short and acrocentric and have satellites on their short arms. The Y-chromosome very closely resembles the chromosomes of this latter group.

There have been two large groups of chromosomal abnormalities found. The first group involved aberrations of somatic chromosomes. The second group is composed of sex chromosome abnormalities. The clinician who is interested in the practical application of chromosome analysis has to decide in what clinical condition this rather intricate and time consuming test is indicated. Our past experience shows that regardless of many questionable chromosome abnormalities reported in a number of isolated cases there are certain clinical conditions in which chromosome aberrations are very likely to be expected. Three types of autosomal chromosome abnormalities have been confirmed to date, each of them causing a specific syndrome namely, mongolism, 13-15 trisomy and 18 trisomy.

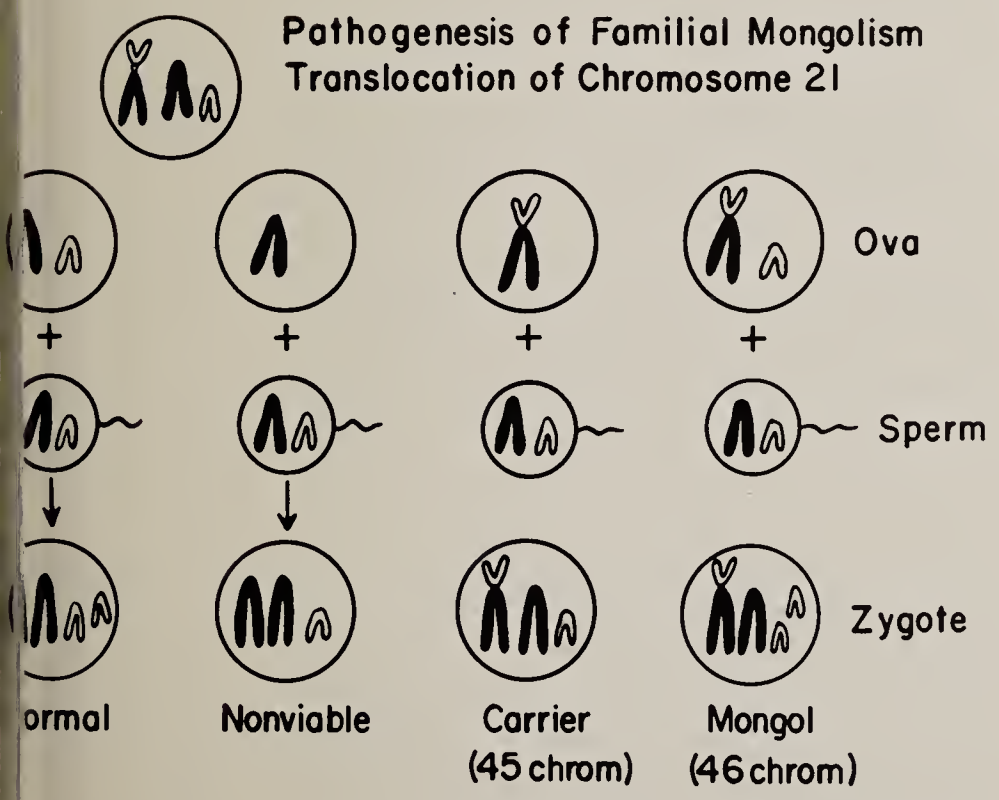

Fig. 1. Pathogenesis of the four types of zygotes in Familial Mongolism with translocation of chromosome No. 21.

ABERRATIONS OF SOMATIC CHROMOSOMES

Mongolism: Lejeune, Gautier and Turpin[5] in 1958 reported that children with mongolism were trisomic for one of the short acrocentric chromosomes—chromosome No. 21. This chromosomal pattern has been found in most of the cases of mongolism reported in the literature. A few exceptions to this have been recorded. In most of these exceptions the chromosome number has been 46 but the pattern is abnormal. The abnormal chromosome found in these cases has been shown to be the result of reciprocal translocation between two short acrocentric chromosomes forming one chromosome resembling the 16-18 group, or between a small and medium-sized acrocentric chromosome. The chromosome No. 21 is represented in each cell in triplicate. This type of chromosome abnormality is found in some cases of familial mongolism. The mothers are usually younger than the mothers of mongols with 47 chromosomes. Chromosome studies of families of mongols with 46 chromosomes revealed that the mothers and some of the female relatives have 45 chromosomes with a 21/15 translocation. These phenotypically normal individuals may have three types of offspring—entirely normal children, phenotypically normal carriers like themselves and classical mongols. (Fig. 1 and 2).

A more unusual form of chromosome abnormality has been encountered in mongolism. In these cases a mosaic state is present in which one cell population carries a normal pair of chromosome No. 21 while the other cell line is trisomic for chromosome No. 21. The clinical diagnosis of mongolism in these cases usually is equivocal and the patients may appear of normal or higher intelligence than the typical mongol. In 1958 Walker[11] published a method describing the use of

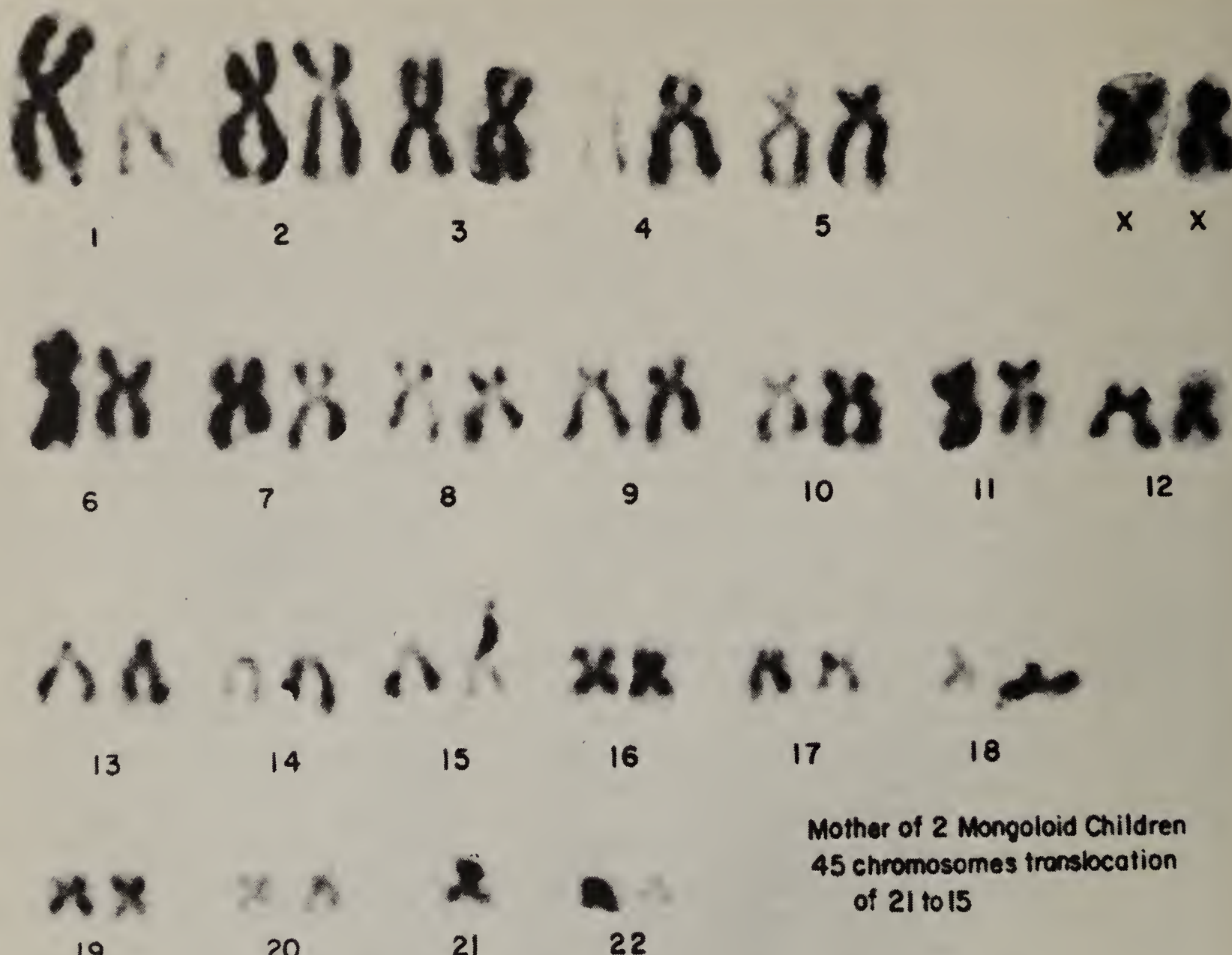

Fig. 2. Karyotype of a mother with two mongoloid children with 21/15 translocation.

dermal configurations of feet and hands in the diagnosis of mongolism. This method uses statistical analysis of the dermal ridge patterns which vary significantly in their frequencies for mongoloids as compared with normal controls. The presence of characteristic dermal ridge pattern in association with clinical stigmata of mongolism thus would establish the diagnosis of mongolism without the help of chromosome analysis. In cases of doubtful clinical findings, recurrence of mongoloid births in the same family and in the younger age group of mothers, chromosome analysis is warranted in order to confirm the diagnosis and to discover the mosaic and the carrier-parent with the greatly increased risk of producing abnormal offspring.

13-15 Trisomy: In 1960 Patau and co-workers[7] first described a patient who had multiple congenital abnormalities associated with a trisomy of chromosomes 13-15. Recently several more cases have been described in the literature. No two cases reported have been identical as to the physical abnormalities present. However, all have been of sufficient similarity to indicate a specific syndrome and to implicate the trisomy 13-15 as etiologically related. The variable degree of abnormal development in this syndrome has been recognized and much remains to be learned of the causal relationship between the trisomy and the phenotypical expression. Mental retardation, failure to thrive, heart defects, eye defects (from complete anophthalmia to colobo-

mata and cataracts), deafness, cleft lip and palate, contractures of fingers, horizontal palmar creases, triangular frontal bone, hemangiomata and seizures have been present in the majority of the reported cases.

18-Trisomy: In 1960 Edwards and associates[1] reported a case of an infant with multiple congenital anomalies and with a trisomy for chromosome No. 17. Simultaneously Patau and his co-workers[9] described two infants with similar abnormalities and with a trisomy for chromosome No. 18. An overall appraisal of the outstanding anomalies in these and subsequently reported cases led to the conclusion that this syndrome was caused by a trisomy for the same chromosome, most likely the chromosome No. 18. The characteristic features of the 18 trisomy include mental retardation, failure to thrive, flexion and deviation of the fingers, micrognathia, defectively ossified short sternum, diaphragmatic hernia or eventration, hypertonia, limited abduction in the hips, dorsiflexion of the short great toe, rocker-bottom feet or pes equinovarus, prominent occiput, malformation of heart and kidneys, umbilical hernia and hypotonia at birth. These findings may be present in the syndrome in various degrees and in many combinations.

It is obvious that in a case of multiple congenital anomalies with mental retardation and failure to thrive chromosome studies may be indicated. The possibility of the existence of other similar syndromes caused by deviations of other chromosome pairs cannot be excluded without investigating large numbers of patients with multiple congenital anomalies.

ABERRATIONS OF SEX CHROMOSOMES

Aberrations of sex chromosomes have been reported in various clinical states. The abnormalities in most cases have been originally suspected after examination of buccal smears for the presence of sex chromatin body. The sex chromatin body, as is well known, is normally confined to the female and is now considered likely to be derived from the condensation of X-chromatin material in the presence of two or more X-chromosomes in the cell. The discrepancy between the nuclear chromatin and the phenotype in sex chromatin positive Klinefelter's syndrome and the sex chromatin negative Turner's syndrome prompted the sex chromosome investigation in these cases. It was discovered that patients with sex chromatin positive Klinefelter's syndrome had 47 chromosomes, the extra chromosome being an X-chromosome. Cases of sex chromatin negative Turner's syndrome had 45 chromosomes, the missing chromosome being X-chromosome. Subsequently, cases of Turner's syndrome and Klinefelter's syndrome were found with other abnormalities on sex chromosomes, including mosaicism. Turner's syndromes with XO/XX, XO/XXX sex chromosome constitution are reported. Klinefelter's syndromes with XXY, XXXY, XXXXY and XYY/XO sex chromosome component are described. Cases of mental retardation in females with or without sexual infantilism have been described with XXX and XXXX sex chromosome complement.

About 43% of phenotypically normal females with primary amenorrhea have been found by Jacobs[4] to have abnormal karyotypes, such as XO, XO/XY, XO/XYY, XO/XXX and XX/Xx. Cases of male sterility in phenotypically normal males may have a sex chromosome complement identical with that of a classical Klinefelter's syndrome. Understature in a female with sexual infantilism with or without mental retardation may have an abnormal sex chromosome constitution. Cases of mental retardation with or without sexual underdevelopment in a female have been reported to have XXX and XXXX sex chromosome pattern.

The clinical problem of intersexuality may or may not be resolved by chromosome analysis. Adreno-genital syndrome in a female can be diagnosed by hormone studies and the presence of normal female buccal smear. In the feminizing testis syndrome the clinical appearance of the

patient, chromatin negative buccal smear and the presence of testicular tissue will establish the diagnosis. In male and female pseudo-hermaphrodites the finding of only one kind of gonad with a compatible buccal smear in most cases will reveal the true nature of the disorder. However, in some cases one may find abnormal chromosome patterns. Shuster and Motulsky in 1962[8] reported a case of a male pseudo-hermaphrodite with a gonad identified as testis, a negative buccal smear and a XX/XY/XO mosaicism. Analysis of chromosomes in true hermaphrodites has revealed several patterns. Hirschhorn[3] in 1961 described a true hermaphrodite in whom XY and XO cells occurred together. Waxman[12] in 1962 reported a case with one ovary and one ovotestis and a questionable XX/XY or XX/Xx mosaicism. Other true hermaphrodites have been described as having XX, XX/XXX and XX/XXx sex chromosome complements. Gartler[2] and associates in 1962 reported a true hermaphrodite with XX/XY pattern in all somatic tissues and two antigenically distinct populations of circulating erythrocytes. The authors concluded that the data are compatible with double fertilization with subsequent fusion of the two genetically different cell masses into a single embryo. It seems apparent that in cases of intersexuality and subnormal sexual development chromosome studies may contribute greatly to the understanding of the nature of the clinical entity.

SUMMARY

The new methods of chromosome analysis have contributed considerably to our understanding of certain pathologic states. In clinical medicine at present, cytogenetic studies are of definite value in selected cases, however, in most instances the diagnosis can be confirmed by more routine methods.

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STUDIES OF HOST-HOMOGRAFT INTERACTIONS

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Transplants of most normal homologous tissues fail to survive in the new host for reasons yet unknown. Ordinarily transplantation of tissues between identical twins has been successful. Mechanisms, immunologic in nature, appear to be involved when transplants are done between hosts of different genetic composition. The following experiments were designed to analyze the destructive mechanisms in host-homograft interactions between members of a genetically impure species. It is recognized that control over these mechanisms is necessary before homografting can be widely practical in man.

In the experimental study of the failure of homografting, several important advantages have been afforded by use of musculofascial tissues from the erector spinae of rabbits. First, resection and implantation of grafts in protected locales could be accomplished with aseptic methods. Secondly, tissues of the graft and host remained distinct so that interactions between graft and host could be accurately interpreted by ordinary microscopic methods. Third, tissues available for grafting were abundant and of uniform composition. Finally, these tissues could lend themselves readily to sterile physico-chemical fractionation, should the need arise.

The freshly resected musculofascial transplant was composed of two different types of tissues. The attached fascia consisted of heavy bundles of collagen between which fibrocytes were dispersed. Delicate strands of collagen passed from the fascia to ensheath the skeletal muscle fibers in secure fashion. The junction between the fascia and the muscle was merely a potential space. However, following transplantation the most distinctive differences between autologous and homologous transplant-host interactions occurred within this junction.

New Zealand rabbits weighing 2.5 to 3 kg. were used. Musculofascial tissues were resected under sterile precautions

From the Division of Pathology and the Laboratory of Surgical Research

These studies were supported by grants H-1630 and 2G-129 from the National Heart Institute, The Muscular Dystrophy Associations of America, Inc., the Otho S. A. Sprague Memorial Institute and the Blind Service Association of Chicago.

from the erector spinae and implanted as autografts and homografts. At the end of the first week, both types of grafts were well healed in place. Thereafter, resorption and organization of both types proceeded so that by the third or fourth week very little of the grafts remained. Two weeks after transplantation was the optimal time for study.

Microscopic study of the autologous musculofascial transplant, two weeks of age, showed a characteristic pattern of degeneration and organization. A bursal sac had formed over the transplant. The floor of the sac consisted of a pannus of vascularized connective tissue which had grown from the host over the fascia of the transplant. The fascia, thickened due to retraction, was heavily permeated by blood vessels arising from the pannus. These vessels penetrated the fascia and ramified as a rich plexus in a zone beneath the fascia created by the gradual absorption of muscle fibers of the graft. (Fig. 1) This zone also contained scattered multinucleated striated cells and many fibroblasts embedded in a newly formed collagenous matrix. Signs of inflammation were minimal. (Fig. 2)

Microscopic examination of the homologous musculofascial transplant, 2 weeks of age, showed similar sequences of degeneration, absorption and organization with the addition of a lymphoid cellular infiltrate which developed principally within the musculofascial zone and spread into the overlying fascia and pannus. (Fig. 3) Angiitis involving newly-formed vascular channels from the host was a frequent accompaniment of the cellular

Fig. 1.—This is a low power photomicrograph of an autologous musculofascial transplant, two weeks of age. A pannus of well-vascularized connective tissue from the host has grown over the fascia of the graft. Between the fascia and the absorbing muscle fibers of the graft is a broad zone containing proliferating connective tissues. This zone is of greatest interest in the evaluation of the type of tissue reaction of the recipient to the transplant. The lateral and inferior margins of the graft are well healed to the tissues of the recipient. X20

infiltration. The numbers of multinucleated striated cells, fibroblasts and newly-formed collagenous fibrils were reduced within the musculofascial zone. (Fig. 4) The classical homologous tissue reaction was a readily reproducible biological phenomenon and served to distinguish the tissues of one animal from those of another of the same species and strain[1].

Fig. 2.—This is a medium power photomicrograph of a portion of the zone illustrated in Figure 1. The empty space above the tissues is a bursa. The floor of the bursa has been formed by a pannus of vascularized granulation tissue from the recipient. The broad zone between the fascia and the absorbing muscle fibers of the graft consists of well-formed capillaries, proliferating fibroblasts and curious multinucleated striated cells embedded in a rich collagenous matrix. Signs of active inflammation are minimal. This is the typical reaction of the animal to its own tissues. X100

Fig. 3—This is a low power photomicrograph of an homologous musculofascial graft, two weeks after transplantation. The pattern of tissue reaction is the same as that illustrated in Figure 1 with the exception of the dense staining of the zone beneath the fascia of the transplant. This is due to the massive accumulation of lymphocytes within this region. The lateral and inferior margins of the graft remain well-approximated to the tissues of the graft bed. X20

On the basis of these observations, it seemed reasonable to assume that autografts and homografts possessed similar capacities to evoke the sequences of autolysis, absorption and stromal organization. The homograft had, in addition, a capacity to stimulate the production of a distinctive type of inflammation. Therefore, further studies, designed to inquire into the origin of this capacity, were made. Homologous and autologous musculofascial grafts were exposed to the effects of low and high temperature and x-irradiation in vitro prior to transplantation. A microscopic study of grafts, two weeks after transplantation, showed deviations from the classical autologous

and homologous tissue reactions. Both types of grafts elicited an indolent fibrocollagenous response from the recipient. The pannus, which had grown over the fascia of the graft, consisted of avascular connective tissue. The fascia showed a loss of cellularity and stained poorly. The musculofascial zone did not develop and the muscle fibers remained in their normal relationship to the fascia. Mechanisms of autolysis and absorption of the graft were delayed. Inflammation was conspicuously absent. (Fig. 5)

Fig. 4.—This is a medium power photomicrograph of a portion of the transplant illustrated in Figure 3. The zone beneath the fascia of the graft contains minimal fibroblastic proliferation and collagen deposition. As the result of the homologous incompatibility reaction, a dense sterile infiltration of lymphocytes has been superimposed upon the matrix of repair within the graft. X60

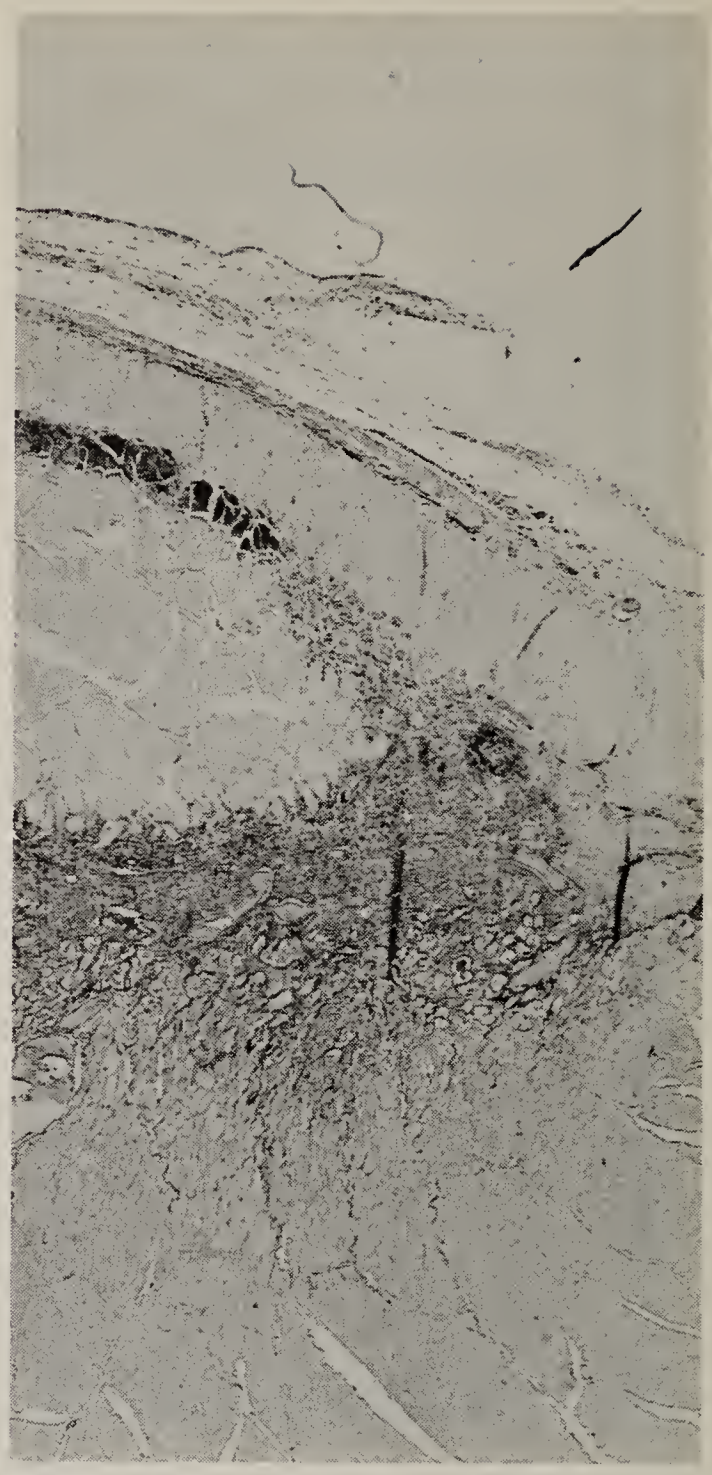

Fig. 5.—This is a low power photomicrograph of an homologous musculofascial graft, 4 weeks following transplantation. This graft had been exposed to -190° C for 25 minutes prior to implantation. The pattern of tissue reaction consists of a fibrocollagenous encapsulation of the graft by the host in the absence of vascular penetration. The usual inflammatory reaction elicited by freshly implanted homologous tissues is strikingly absent. The dark staining of the muscle fibers beneath the fascia of the graft is due to pathologic calcification which is observed, at times, in both autologous and homologous musculofascial grafts. The absorption of skeletal fibers of the graft is taking place principally at the lateral and inferior margins of the transplant. Proliferating admixtures of cells from graft and recipient are necessary to elicit the customary homologous inflammatory reaction. X20

Fig. 7.—This photograph shows two rabbits following anastomosis of their respective ears. Plaster casts about the neck and upper thorax have been joined together with adhesive tape. In three to four days following anastomosis, cross-circulation of blood was established. This continued to about 13 days after union at which time the cross-circulation was terminated due to the effects of the incompatibility reaction between the opposed homologous tissues. (See Fig. 8)

Following less extreme exposure to temperature and x-irradiation, grafts showed variations of the classical pattern of organization with an associated inflammatory reaction of varying degree in homografts. The amount of admixture of proliferating cells of the graft with those of the host seemed to determine the magnitude of the inflammatory reaction in and about the homograft. (Fig. 6) This raised the question of the possibility of the extent to which "viability" of cells of the graft was necessary in exciting and maintaining the normal autograft-host and the abnormal homograft-host interactions[2,3].

To obviate changes brought about by the resection and temporary isolation of tissues, a method was developed whereby microscopic observations could be made at the sites of surgical union between homologous tissues, each possessing its own blood supply[4]. Rabbits surgically united together by their ears initially showed normal sequences of healing. (Fig. 7) Within three to four days, dermal epithelium became continuous and a cross-circulation was established between

Fig. 6.—This is a low power photomicrograph of an homologous musculofascial transplant, two weeks of age. Prior to transplantation, the right half of the graft was exposed to 500 "r" of x-irradiation. The left half of the graft was protected with lead shields. The protected half shows the typical pattern of homologous tissue reaction accompanied by the usual inflammatory reaction. In the right half of the graft, which had been irradiated, the incompatibility reaction has failed to develop. The subjacent skeletal muscle fibers show no unusual morphological changes though mechanisms of autolysis and absorption have been delayed as a result of the irradiation. Cellular injury to the graft by irradiation has completely eliminated the inflammatory stimulus normally residing within untreated homografts. X20

parabionts. At six or seven days an inflammatory reaction which gradually increased with the passage of time developed at the site of anastomosis so that cross-circulation between parabionts was terminated within 13 days following surgical union. The ears remained firmly attached to one another in spite of the ischemia and inflammation because the matrix of collagen and reticulum mutually deposited during the early phases of healing remained "immune" to the destructive effects of the homologous incompatibility reaction. (Fig. 8)

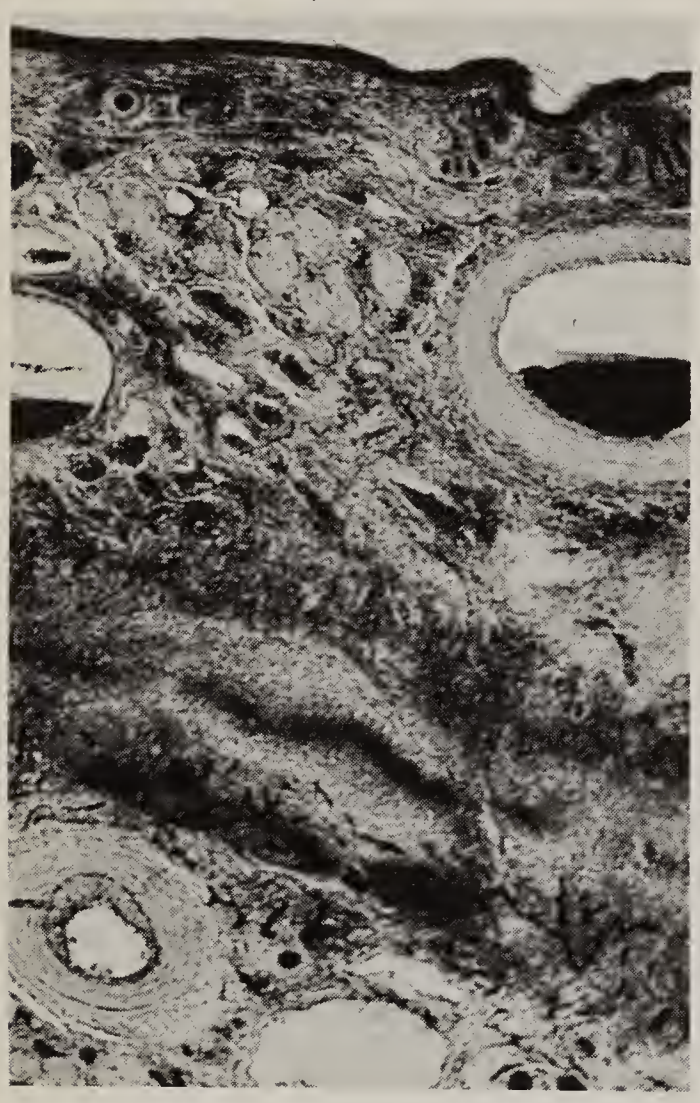

Fig. 8—This is a medium power photomicrograph of the central region of a homologous aural junction, three weeks after anastomosis. The epithelial surfaces of the parabionts are normal. In the transverse plane of the illustration, a well demarcated inflammatory reaction has developed. The blood vessels of the tissues of the parabiont occupying the lower half of the illustration have been filled with India ink. Ink has not crossed the junction into vessels of the uninjected parabiont. Cross-circulation was never demonstrated at this age of union. A second aural union of the parabionts resulted in a spontaneous separation of the ears by the 5th or 6th day following anastomosis. Cross-circulation was never established during the second period of parabiosis. X40

In confirmation of the well known second set homograft rejection phenomenon[5], rabbit parabionts joined together a second time failed to reproduce sequences of healing noted at primary aural junctions. The second aural junction healed poorly and usually underwent spontaneous separation by the fifth or sixth postoperative day. This observation led to the belief that a systemic alteration of immunologic nature had occurred in parabionts as a result of the initial surgical union. To further characterize this alteration, postparabiotic partners were cross-grafted with musculofascial grafts. This resulted in a curious modification of the classical homologous tissue reaction. Two weeks following cross-grafting the homografts were well healed in place. (Fig. 9) Microscopic

Fig. 9.—This is a photograph illustrating the gross appearance of homologous musculofascial grafts two weeks after implantation in the postparabiotic recipient. The graft at the right side of the illustration was resected from a random adult control donor. A pink-streaked pannus of recipient's connective tissues containing many vascular channels has grown over the fascia of the graft. This is the normal appearance of both autografts and homografts. The graft on the left side of the illustration was resected from the parabiotic twin. The fascia of the cross-graft remains white in appearance due to the production by the recipient of a translucent layer of avascular connective tissue. Parabionts, in most instances, were capable of distinguishing between their respective partner's tissues and those of random control animals of the same species.

examination revealed two types of tissue reaction, equally distributed between the postparabionts[6]. The first type consisted of a fibro-collagenous encapsulation of the graft in the absence of vascular stromal penetration by the recipient. The architecture of the graft remained unchanged. Inflammation was absent. (Fig. 10) The second type of post-parabiotic modification was similar to the first with the addition of a peculiar angiomatous plexus of capillaries arising from the recipient and ramifying beneath the fascia of the graft. In spite of this rich vascularization by the host, absorption of the graft was delayed. Also, fibroblastic proliferation and collagen deposition were minimal. Inflammation was again absent. (Fig. 11)

Since cross-circulation, though transient in nature, was a conspicuous feature of parabiosis, experiments involving the cross-transfusions of whole blood, controlled in quantity, were done[7]. This eliminated all factors involved in parabiosis, except exchange of blood. Seven to 10 days following exchange blood transfusions of 200 to 360 ml, the cross-transfused pairs were cross-grafted with musculofascial tissues. Two weeks later, microscopic analysis showed a vascularizing stromal penetration identical to the second type of host-homograft interaction described as following parabiosis. (Fig. 11) The post-transfusion homologous tissue reaction was reproducible and appeared to bear no relation to the known blood groups in rabbits.

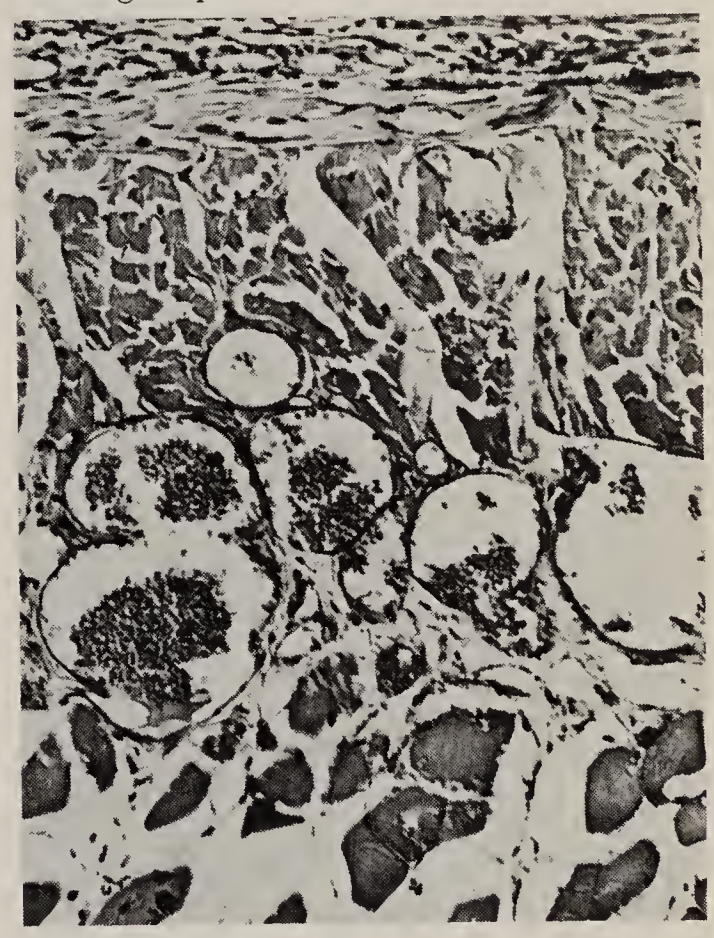

Fig. 10.—This is a medium power photomicrograph of a part of a postparabiotic musculofascial transplant, two weeks after implantation in the postparabiotic recipient. The graft is covered by avascular connective tissues which have originated from the recipient. The fascia of the graft, though cellular, remains free of vascular penetration. The customary zone between the fascia and the muscle fibers has failed to develop. Inflammation is absent. This tissue reaction represents the least active of the microscopic host-homograft interactions among the cross-transplants made between separated parabiotic twins. X325

Fig. 11.—This is a medium power photomicrograph of a part of a postparabiotic transplant, two weeks after implantation into the postparabiotic recipient. A well-vascularized pannus of connective tissues overlies the graft. The fascia contains many vascular channels which terminate in a peculiar angiomatous pattern within the zone between the fascia and skeletal muscle fibers. In spite of this intense vascularization, the customary features of the homologous incompatibility reaction have not developed. Inflammation is absent. Also, autolysis and absorption of skeletal muscle fibers have been delayed. This tissue reaction represents the second type of postparabiotic host-homograft interaction and seemed to bear no relation to the length of time of the parabiotic period. X325

A subsequent series of experiments demonstrated that the leukocytic fraction of the blood contained the conditioning mechanisms responsible for altering the local reaction of the recipient to the homograft resected from the donor of the leukocytes. Lymphocytes from the peripheral blood were isolated in high purity by the Ottesen technique[8]. (Fig. 12) Paired rabbits were cross-transfused with lymphocytes and then cross-grafted. Two weeks later, microscopic observation revealed modified homologous tissue reactions identical to those observed following parabiosis and transfusions of whole blood. (Fig. 11) A minute quantity of lymphocytes (10^5 or 10^6) was all that was required.

Further studies involving the desiccation and disruption of lymphocytes showed that conditioning mechanisms present in fresh lymphocytes were lost following such treatment. Recipients of lymphocytes so treated exhibited classical homologous tissue reactions following transplantation. These studies indicated that a high degree of cellular integrity was required in order to "immunize" the recipient in such a way as to elicit the described changes in the reaction pattern to homografts[9,10].

It was soon learned that the relationship between donor and recipient was not necessarily a specific one in terms of host-homograft interactions. Approximately one-third of homologous musculofascial grafts when implanted in postparabiotic recipients from control random donors elicited a tissue reaction identical to that observed between specific donor and recipient. Further confirmation of the non-specificity of modified homologous tissue reactions, became apparent in the study of adult recipients receiving pooled blood transfusions from donors other than the donors of the grafts. In this instance, by transfusing blood from six donors into a single recipient prior to grafting, the percentage of post-transfusion homologous tissue reactions could be raised to 80 per cent[11].

Fig. 12.—This is a photograph illustrating the Ottesen apparatus used in separating lymphocytes from whole blood. The centrifuge tube contains a lucite cylinder with a central hollow column. A lucite tear drop occupies the bottom of the tube. Following centrifugation at 2000 rpm at +18° C, the lymphocytes are forced up through the center column within the cylinder as shown in the illustration. Lymphocytes of 98% purity were obtained in this manner.

It was also determined that injection of either pooled embryonic or placental tissues (7-11 per litter) into a single nonpregnant host prior to random grafting of adult musculofascial tissues caused a host-homograft interaction identical to that observed in hosts receiving pooled blood transfusions prior to grafting from a random donor[12,13]. (Fig. 11)

Further experiments were done to determine whether or not other somatic tissues than circulating leukocytes possessed conditioning mechanisms capable of altering the reaction of the recipient to homologous musculofascial tissues. Rabbits were cross-grafted with full thickness skin grafts. Two weeks later, the same hosts exchanged musculofascial grafts. Two weeks after the last grafting procedure, microscopic examination of the musculofascial grafts revealed a modified pattern of homologous tissue reaction[14]. An identical situation oc-

curred if the hosts were pretreated with single grafts of musculofascial tissues prior to grafting with the same type of tissue. Pretreatment of the host with suspensions of graft donor's hepatic cells have led to equivocal results either on the basis of hemic contamination of the hepatic cells or loss of viability of hepatic cells prior to injection[15]. However, blood-free preparations of graft donor's retinal cells have failed to immunize the recipient as indicated by the appearance of classical homologous tissue reactions following pretreatment of recipients with graft donor's retinal cells prior to grafting. In these experiments the question of maintenance of viability of the retinal cells following isolation has remained unsolved[16]. Isolation of other somatic tissues, free from blood leukocytes, still remains important in the issue as to whether or not all nucleated cells of the host contain, in common, the conditioning mechanisms necessary to provoke the modified homologous tissue reaction.

In an attempt to explain the modified homologous tissue reaction, it seemed reasonable to assume that the sequences of healing in and about the homograft in the pretreated recipient were so determined by an accelerated rate of cellular injury to the graft and that the pattern of tissue reaction represented a state of enhanced antagonism between homograft and treated host. In support of this, experiments have indicated that the modified homologous tissue reaction can be obtained if the graft is implanted in the original donor following six days of residence in the pretreated recipient. On the other hand, the autologous tissue reaction is seen when the graft is returned to the donor following temporary residence in the untreated recipient[17].

In view of some similarity between parabiosis and pregnancy in which vascularized fetal and maternal tissues remain in apposition to one another, a study of the homologous musculofascial tissue reaction was made in the pregnant host. Gravid rabbits (13 to 15 days of gestation) were cross-grafted with non-pregnant females and males of the same species. Autografts were simultaneously implanted. Two weeks later, the animals were killed and the grafts prepared for microscopic study. Each gravid host contained eight or more normal fetuses. The pregnant host reacted in an identical manner to autografts and homografts. Both types of grafts showed the usual sequences of degeneration, absorption and organization characteristic of host-autograft reactions. (Fig. 13) On the other hand, homografts of tissues from pregnant donors in non-pregnant hosts showed varying degrees of the classicial host-homograft reaction with conspicuous inflammatory response[18].

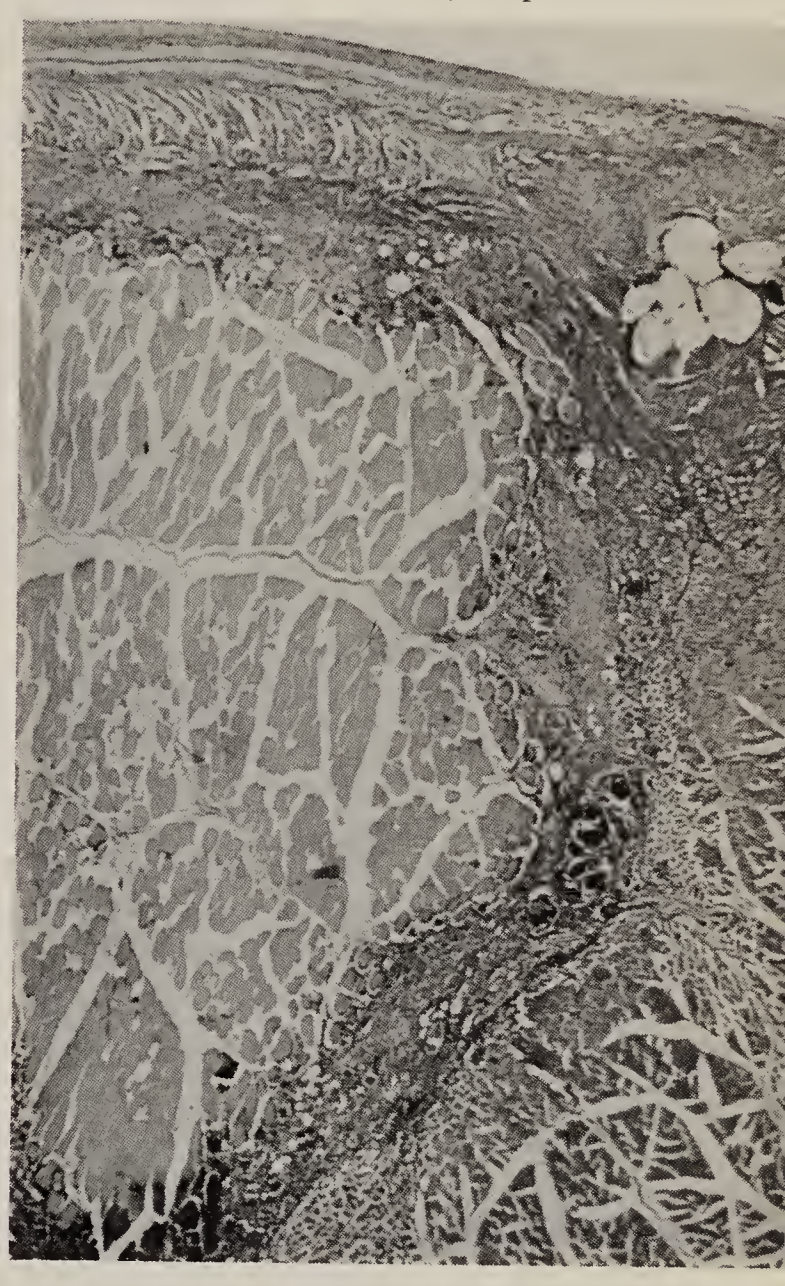

Fig. 13.—This is a low power photomicrograph of an homologous musculofascial transplant, two weeks after implantation in the normal pregnant host. Note that the tissue reaction is identical to the pattern of reaction to primary homologous grafts in non-pregnant recipients except for the conspicuous absence of inflammation. In this unusual instance, sequences of healing have proceeded as if the foreign tissue were native to the maternal host.

Since the initial response of the pregnant host to homografts was a harmonious one, a series of experiments were done in which the pregnant host received graft donor's leukocytes prior to grafting. The homograft reaction in the pretreated pregnant host was identical to that observed in non-pregnant recipients pretreated with parabiosis, blood transfusions or leukocytic transfusion. (Fig. 14) It was concluded that alteration of the reactivity of the pregnant host could always be achieved by transfusion of leukocytes prior to grafting in much the same manner as non-pregnant hosts[19].

This reactive capacity of the pregnant host to homologous "tissue immunization" was also demonstrated in the human. An accelerated rejection of adult human skin grafts was obtained when the same host was grafted from the same donor during a subsequent pregnancy, 2.5 years after the initial grafting procedure[20].

Since embryonic tissues as well as placental tissues possessed conditioning mechanisms capable of altering the homologous tissue reaction in non-pregnant hosts and the fact that the pregnant host could be influenced by injection of adult leukocytes prior to grafting, experiments involving utero-placental trauma were performed at mid-gestation. The pregnant host then received a musculofascial graft resected from an adult random donor. (One pregnant rabbit died immediately after the trauma. Microscopic examination showed groups of cells resembling those at the utero-placental junction lodged within the pulmonary arteries.) Two weeks later the pregnant rabbits which had received utero-placental trauma prior to grafting were killed. Microscopic examination of the homografts showed that accelerated graft injury occurred in 25 per cent. These studies indicated that it was possible by the production of utero-placental trauma prior to grafting to alter the local reaction of the pregnant host to homografts of adult tissues[21].

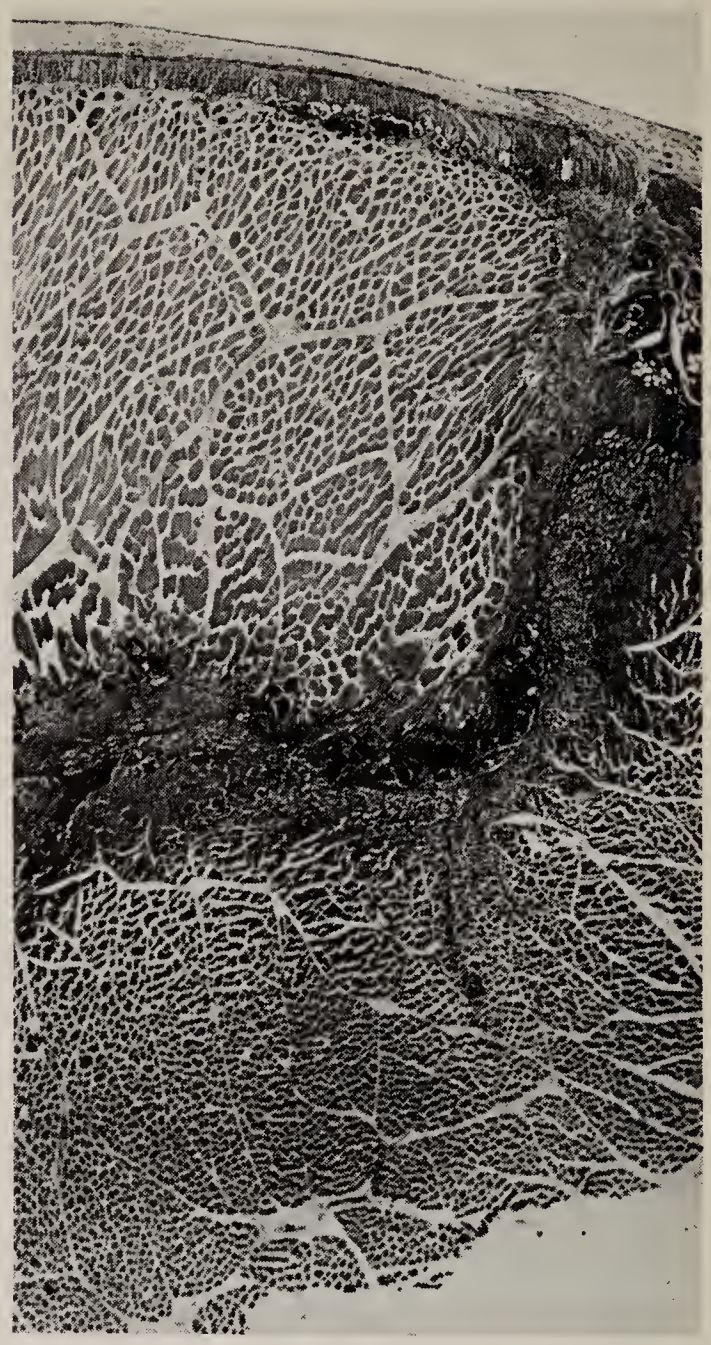

Fig. 14.—This is a low power photomicrograph of an homologous musculofascial transplant, two weeks after implantation in the pregnant recipient which had been transfused with graft donor's leukocytes prior to grafting. This is the modified homologous tissue reaction which illustrates the reactive capacity of the recipient to "homologous immunization" during pregnancy. Compare with Figure 11 of the non-pregnant recipient. X20

Since the pregnant host was capable of enhancing mechanisms injurious to homografts of adult musculofascial tissues, experiments were designed to determine whether or not it was possible to quicken the normal mechanisms of degeneration of utero-placental tissues by challenging the maternal host at mid-gestation with fresh blood from multiple unrelated male donors. Each maternal host received 2.5 ml of whole blood from each of ten donors. Litter size was determined in each host by direct observa-

tion. Two weeks later, the animals were killed. All fetuses were normal with no evidence of runting syndrome. Microscopic examination of the utero-placental sites showed no unusual changes. These studies indicated that it was not possible to disturb prematurely the natural mechanisms of deterioration of utero-placental tissues by alerting mechanisms which accelerate injury to homografts of adult tissues[22].

Though the aforementioned phenomena are without explanation, these studies provide a basis for further inquiry into not only the incompatibility reactions of adult hosts to adult homografts but also those involving fetal-maternal relationships.

The authors wish to thank Mr. J. deBruin, Director of the Laboratory of Medical Illustration and his staff for preparing illustrations reproduced in this review.

The microscopic sections were all stained with hematoxylin and eosin.

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SUMMARY

Autologous and homologous musculofascial tissues of rabbits undergo similar sequences of degeneration, absorption and organization following transplantation with exception of the homograft in which a characteristic sterile lymphocytic infiltrate is superimposed upon the sequences of repair within the homograft. The stimulus for inflammation within the homograft appears to be related to the degree of admixture of proliferating cells of graft and host. Interference with this characteristic of the host-homograft interaction by detrimental in vitro treatment of the graft prior to implantation diminishes or eliminates the inflammatory response.

Pretreatment of the recipient by parabiosis, whole blood transfusions, leukocytes, skin, embryonic tissues, placental tissues and musculofascial tissues results in a reproducible modification of the classical homologous musculofascial tissue reaction. Fibro-encapsulation of the homograft with varying degrees of vascular penetration occurs in the absence of the customary lymphocytic infiltrate. The modified homologous tissue reaction is viewed as an expression of enhanced antagonism between graft and host. Decreased viability of the graft in "immunized" hosts occurs prior to the vascularizing period (7 days) for musculofascial grafts. A freely diffusable substance or group of substances independent of cell transfer appear to be implicated in the alteration of the host-homograft interaction in the "immunized host."

The study of the behavior of homografts in the pregnant host has shown that, although the pregnant host reacts initially to homografts in the same manner as to autografts, pretreatment of the pregnant host with viable homologous cells results in "tissue immunization" as indicated by the occurrence of the modified tissue reaction to homografts. Embryonic tissues and placental tissues also contain conditioning mechanisms capable of eliciting enhanced antagonism to homografts in pretreated non-pregnant recipients. In addition, utero-placental trauma at mid-gestation sufficient to produce escape of cells from the utero-placental sites was capable of altering the local reaction of the pregnant host to homografts of adult tissues.

The capacity of the pregnant host to react to the challenge of homologous tissues has been demonstrated at the clinical level. The human pregnant host, when skin grafted from the same donor for a second time during a subsequent pregnancy, has shown the accelerated second set rejection phenomenon. This has revealed a most interesting degree of reactiveness of the "tissue immune" system of the human pregnant host against homografts of adult tissues.

Further studies are required to understand the nature of the signal which elicits inflammation to homografts. Also, the mechanisms of antagonism of the "tissue immunized" host need clarification. Lastly, it is important to determine whether or not an abnormal escape of cells of fetal origin into the maternal circulation can influence unfavorably the respective gestational site.

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ABSTRACTS AND REVIEWS

OF PUBLICATIONS BY THE STAFF

Erythrocythemia. M. A. Escobar, F. E. Trobaugh, Jr.: *Med. Clin. of North Am., 46:1, January, 1962.*

A review of erythrocythemia and its relationship to polycythemia rubra vera and to stress erythrocythemia is discussed. Erythrocythemia is characterized by an elevation in the peripheral red cell count and hemoglobin concentration, an increased red cell mass, normal numbers of white blood cells and platelets and no splenomegaly. The bone marrow shows erythroid hyperplasia. It seems always to be secondary to a disease state or environmental abnormality such as hypoxemia, renal disease, brain disease and others. It is usually reversible by correction of the primary disorder. Erythrocythemia is to be distinguished from polycythemia and stress erythrocythemia. Polycythemia is a primary disease of unknown etiology in which there is an increase in total circulating red cell mass and all of the formed elements in the blood, generalized bone marrow hyperplasia and splenomegaly. In stress erythrocythemia there is plethora, a diminished plasma volume and a normal red cell mass. The white cell count, platelet count and bone marrow activity are normal. Splenomegaly is absent and a mild hypertension is usually present. A summary of diagnostic features and pertinent case histories are included. A thorough review is made of the abnormalities which result in erythrocythemia. The role of erythropoietin is discussed.

Systemic Lupus Erythematosus and Pregnancy. M. Garsenstein, E. Pollak and R.M. Kark. *The New England Jour. of Med. 267:4, pp. 165-169; July 26, 1962.*

Data on 33 pregnancies in 21 patients ill with systemic lupus erythematosus were reported. Nineteen pregnancies resulted in live births, but two infants died in the immediate neonatal period.

Exacerbations of systemic lupus erythematosus occurred with particular frequency in the first eight weeks after delivery. The risk of an exacerbation at this time was seven times as great as in appropriate control periods in the same patients.

Four of the 21 patients died. All had histologic evidence of severe renal involvement, and all were associated with evidence of active systemic lupus erythematosus in the puerperal period.

Carcinoma of the Colon. W. H. Cole, S. Economou and J. Hunter. *The Surg. Clin. N. America, 42:2, April, 1962.*

Cancer of the colon is one of our most common carcinomas, and, fortunately, is one of the most favorable from the standpoint of cure.

Improvement in techniques and precautions at the time of surgery influences these results. Local Recurrence: Linen ties inside the line of resection, rinsing the bowel lumen locally with anti-cancer agents and trimming the crushed edge of bowel prior to anastomosis has markedly reduced suture line recurrence. Venous Emboli: Minimal handling of tumor and early ligation of vascular trunks supplying the tumor area may favorably influence the cure rate. Adjuvant Chemotherapy: Despite the lack of improvement in cure rate of colon cancer with such therapy it appears promising with other cancers and other chemotherapeutic agents. The authors believe that surgery plus a chemotherapeutic agent is superior to surgery alone.

Aortic-Cardiacfistulas Following Corrective Operations for Ventricular Septal Defects and Tetralogy of Fallot. M. H. Agustsson, M. Weinberg, Jr., B. M. Gasul, E. H. Fell, J. P. Bicoff, Z. Steiger, R. Arcilla, and T. Iwa. *The J. of Thoracic and Cardiovascular Surgery, 43:166, 1962.*

Following surgical correction of ventricular septal defects and tetralogy of Fallot in five patients, two cases of aortic insufficiency, two sinus of Valsalva-right ventricular fistulas, and one coronary artery-right ventricular fistula developed as postoperative complications. One patient died in congestive heart failure six hours after operation, two developed heart failure and murmurs suggestive of fistulas 10 days after operation, and two developed continuous murmurs without symptoms several months after operation. In the surviving patients, the defects were demonstrated by retrograde aortography and were subsequently repaired successfully.

The following points are emphasized: 1. The aortic valves and sinuses are adjacent to the superior borders of most ventricular septal defects and may be injured in corrective operations. 2. The defects may be unrecognized causes of congestive heart failure and death in the immediate post-operative period, or may be missed later if careful follow-up is not maintained. The characteristic diastolic or continuous murmurs should suggest these diagnostic possibilities. 3. Retrograde aortography is the procedure of choice in establishing a definitive diagnosis. 4. Reoperation is mandatory for survival in those patients in heart failure due to an acquired aortic-cardiac fistula, and should be undertaken as soon as the diagnosis is established.

Elastic Properties of Single Elastic Fibers. R. W. Carton, J. Dainauskas and J. W. Clark. *J. Appl. Physiol. 17:3, pp. 547-551, 1962.*

The elastic properties of elastic tissue were studied in a situation which minimized the effects of extraneous connective tissue and of the position of fibers in the elastic network. Single elastic fibers were dissected free from the ligamentum nuchae of the ox and were stretched under conditions of constant temperature and salinity. The strain was an exponential function of the applied tension. Single fibers were found somewhat less stretchable than the ligaments from which they were taken. The data given can be used to calculate the contribution of such elastic fibers to the behavior of an elastic system in which they are incorporated.

Epiphyseal Stapling. A. Trias, K. Mueller and R. D. Ray. *Surg. Gyn. & Obst. 113, pp. 315-323, Sept., 1961.*

Leg length inequality is discussed on the basis of history of the problem, methods of controlling the condition and in particular, the details concerning the stapling technique for temporary growth arrest.

Complications have been high in the 17 cases reviewed and these have been traceable to errors in pre-operative planning, in surgical technique and in post-operative care. Pre-operative planning includes determination of the type of shortening, that is, whether it is functional or structural. A prediction of growth potential is necessary on the basis of several tables available.

Leg lengths are determined by teleoroentgenograms and the use of "bone age" rather than the chronological age. Planning may direct that bone shortening osteotomies be done instead of epiphyseal arrest.

Surgical technique is given in detail and the common obstacles found in the stapling procedure are discussed with suggestions to avoid complications.

Post-operative care is quite important in that close follow-up is needed to prevent new deformities, to detect broken or displaced staples and to cope with growth spurts.

The effectiveness of staples depends on the rate of growth of the opposite or short leg. This fact alone dictates the length of time the staples must be left in place, barring complications.

The Effect of a Single Intravenous Injection of Aminonucleoside of Puromycin on the Rat Kidney: A Light- and Electron-Microscope Study. R. Lannigan, R. M. Kark and E. Pollak. *The Jour. of Pathol. and Bacteriol. 2, pp. 357-362, April, 1962.*

In the rat a single intravenous injection of aminonucleoside of Puromycin (10 mg. per 100 g. body weight) produced within 3 days a significant proteinuria, which persisted for 143 days. Periodic acid-Schiff-positive droplets were concurrently found in glomerular ephithelial cells and in cells of proximal convoluted tubules. Casts were prominent and increased in number during the course of the experiment.

Electron-microscope studies showed broadening of the foot processes at 24 hours and replacement by a continuous layer of epithelial cytoplasm in some glomeruli at 8 days. The epithelial cells were swollen, vacuolated and contained osmophilic droplets in their cytoplasm. Loss of the foot process layer was still noted at 112 days. Proliferative changes and thickening of the basement membrane were not observed.

The Role of Regional Node Dissection in the Treatment of Malignant Melanoma. H. W. Southwick, D. P. Slaughter, J. F. Hinkamp and F. E. Johnson. *A.M.A. Arch. Surg. 85, pp. 80-86, July, 1962.*

Presented are 245 patients with a diagnosis of malignant melanoma. Excluding those patients who are indeterminate, who received only palliative treatment, or who were operated upon since Jan. 1, 1956, there are 142 patients for evaluation. The over-all 5-year survival free of disease is 37.4%.

The most significant group for evaluation is the 96 patients whose regional nodes were without clinical evidence of metastatic disease when first seen.

Seventy-one of these patients have had local treatment followed in 29 instances by a therapeutic node dissection. Twenty-three of these (32.4%) were living free of disease at 5 years.

Twenty-five patients had either an elective excision and dissection in continuity or local excision followed by elective node dissection. Twenty of these patients (80%) are living free of disease at 5 years.

Elective regional node dissection is recommended as part of the primary definitive treatment of malignant melanoma.

Use of Fluorescein-labeled Fibrin for the Determination of Fibrinolytic Activity. R. Paggenhagen, J. L. Koppel and J. W. Olwin. *J. Lab and Clin. Med. 59:5, pp. 1039-1046, June, 1962.*

A simple and rapid method for the preparation of fluorescein-labeled human fibrinogen has been described together with a procedure for its use in the measurement of fibrinolytic activity. Data has been presented to show the applicability of this assay method to the quantitative determination in purified systems of plasminogen concentration, plasminogen activator activity, plasminogen activator inhibition, and the inhibition of plasmin activity per se. The translation of these procedures to the measurement of the same components of the fibrinolytic system present in biologic media, such as plasma and tissue extracts, has been discussed.

Symptoms and Signs of Peripheral Arterial Disease. G. de Takats. *Med. Clin. N. Amer. 46:3, May, 1962.*

Diabetes, gout, hypertension and lipemia seem to accelerate the process. Angiograms reveal narrowing or occlusion at segments subjected to localized trauma, such as the posterior wall of the abdominal aorta at the bifurcation, just peripheral to bifurcations, at the upper end of Hunter's canal or over the soleus tendon. Pulses are diminished,

absent or, if present, may show a delayed pulse wave coming in through collaterals. Pulses may disappear on exercise, together with a drop in skin temperature.

The diabetic angiopathy is not just accelerated atherosclerosis. The defect in the venous walls causes micro-aneurysms, visible in the retina and as subcutaneous hemorrhages in the toes at the slightest provocation. The tourniquet test is usually positive for capillary fragility. The neuropathy allows the development of painless ulcers and patches of gangrene, facilitating rapidly ascending lymphangitis. Complete survey of heart and vascular tree is obligatory before recommending treatment.

The Surgical Treatment of Diabetic Vascular Disease. G. DE TAKATS. *Ill. Med. J., June, 1962.*

There is no single measure of proper management of diabetic neurovascular disease. Proper foot hygiene, including antiseptic soap, skin creme and chiropody eliminate the necessity of foot amputation in many instances. Incisions and minor amputations are often possible if arteriolar involvement is not too pronounced. Sympathectomies are useful 1) if there is demonstrable sympathetic activity with somatic nerve involvement, 2) if the level of amputation below the knee is questionable. Sympathectomies are useless in the dry, sympathectomized feet and in the presence of neurotrophic joints. Major amputations should always be done in an attempt to save the knee joint, if the patient will be able to use an artificial leg. Prophylactic sympathectomy of the other leg should be considered. Direct arterial surgery only occasionally is indicated.

The Interrelation of Fats, Blood Coagulation and Atherosclerosis. J. L. KOPPEL and J. H. OLWIN. *Heart Bulletin, 11:4, Page 78, July, August, 1962.*

The part played by excessive fat intake and abnormal fat metabolism in the development of atherosclerosis has been emphasized by many authorities over a period of years. However, the influence of fats on the coagulation mechanism and the interrelation of thromboembolism and atherogenesis (suggested by Rokitansky over a century ago) are less well known. This paper deals with the biochemical aspects of these interrelationships both in vivo and vitro. The observed influence of different types as well as amounts of lipids on the various stages, factors, and overall process of the coagulation mechanism are reported, and these effects are discussed as they may be related to various aspects of thromboembolism and atherosclerosis. Related clinical observations and data are reported and correlations with the work in the basic fields are suggested.

Isolated Circular Intestinal Loop. R. E. ANDERSON, W. E. DIFFENBAUGH and W. H. SCHMIDTKE. *A.M.A. Arch. Surg. 84: pp. 559-563, May, 1962.*

To obviate some of the disadvantages of the several intestinal fistulae which are commonly utilized for the experimental study of intestinal function, a different type of preparation has been developed. Based upon the concept that continuous peristaltic activity is the most fundamental of the normal activities of the bowel and that precise conclusions as to physiological or pathological phenomenon must be based upon the completed cycle of the specific event being considered has motivated the design of a completely circular loop of intestine with an afferent conduit.

Such a loop is simply prepared by isolating the desired length of intestine, undertaking an end-to-side anastomosis upon it and bringing the proximal end through the anterior abdominal wall as an access fistula. The final segment, by such a procedure, has the appearance of the figure "6." Restoration of the normal flow in the remaining gut is established by primary end-to-end anastomosis.

This preparation offers definite advantages over those currently being used. There is complete continuity in the isolated bowel, as well as in the remaining intestine. Stagnation and obstructive reflexes are therefore eliminated. Material placed into such

a loop will remain in a normal or altered intestinal environment for an indefinite period of time until absorption, neutralization, elimination or a combination of these processes has taken place by whatever means the host would otherwise accomplish this at that particular area in the intestinal canal. Absorption and secretion ratios remain constant as the intraluminal contents circulate for an indefinite period of time.

The Effect of Drugs on the Incorporation of Labeled Precursors into DNA and RNA in Malignant Cells. G. O. McDonald, A. N. Stroud, A. M. Brues and B. R. Svoboda. *Proc. Am. Assoc. for Cancer Res. 3:342, 1962.*

Little is known about the incorporation of tritiated thymidine into DNA and tritiated uridine into RNA of malignant cells *in vitro* after treatment with the drugs HN_2, 5FU, actinomycin D and TSPA. Chemotherapy experiments were designed to look for effects of these drugs on DNA and RNA synthesis which might eventually result in growth inhibition or cell death.

Our results suggest that certain of these drugs block the *de novo* synthesis of thymidine or its precursors, resulting in increased utilization of the exogenous labeled thymidine for DNA synthesis, as indicated by increased grain counts. This effect differs between the tumors tested, and was greatest and most consistent with 5FU in the dosage utilized. Sister cells exposed to uridine for 1-24 hours show labeling throughout the cell (nucleolus, nucleus and cytoplasm), except in the case of 5FU and actinomycin D. In the 5FU-treated cells the label was concentrated on nucleoli only, whereas part of the actinomycin cells were labeled in nucleoli only, and part had label throughout the entire cell. It would appear that 5FU and actinomycin D block or delay chromatin RNA synthesis and not nucleolar RNA synthesis.

A Method for Studying Fe Absorption Using Ferrous^{59} Sulfate Tablets. M. A. Escobar, A. Emanueli and F. E. Trobaugh, Jr.: *J. of Nuc. Med. 3: 3, May, 1962.*

Intestinal absorption of radioactive iron has been studied by measuring the incorporation of orally administered Fe^{59} into the circulating red blood cells. Two different pharmaceutical preparations of ferrous sulfate were tested. Iron deficient patients and control subjects with normal serum iron and peripheral red blood cells were studied. Two tests were performed on each subject in an attempt to provide measures of within subject variation and any residual effects. Each subject received two test doses in the order selected from a prepared table of randomization. It was assumed that the rate of erythrocyte incorporation of plasma iron would remain constant in the same individual during the four week period of the experiment. Any difference noted in the red blood cell incorporation of orally administered iron between different tests on the same subject were assumed to be due to differences in intestinal absorption. The red cell mass of each individual was determined using Cr^{51} sodium chromate. One hundred and five mg of ferrous sulfate containing 25 microcuries or less of Fe^{59} were administered orally to a fasting subject. Fe^{59} activity of the peripheral blood was determined three times weekly until the plateau of iron incorporation in red blood cells was reached. At that time the proportion of the administered iron absorbed was determined by referring the total circulating Fe^{59} to the amount administered. Twenty-one patients and twenty controls were studied.

Details of the technique, including the selection of subjects and the corrections made for variation of isotope content between tablets are presented.

Methods of Potentiating the Action of Sulfhydryl Inhibitors Against Human Cancer Cells. F. E. Knock, *Surg. Gynec. Obst. 114:2 pp. 189-20, 1962.*

The action of a number of known sulfhydryl inhibitors, tested *in vitro* against 3 human tumor cell lines (HeLa, KB, and HEP#2), and tumor specimens removed at surgery,

was potentiated by fluoride, malonate, urea, surface-active agents, and diathermy and was decreased by X-Ray. The same group of chemicals tested against mouse Ehrlich ascites cells showed no potentiation of the sulfhydryl inhibitor activity alone, nor was the combined action enhanced by diathermy. In tests of a variety of chemicals against human cancer cells removed at operation, great variability in effect was seen from tumor to tumor.

Complications of Gastro-Intestinal Intubation Collective Review. C. Hanselman and H. Meyer. *Surg., Gynecol. and Obst. March, 1962.*

The literature on gastro-intestinal intubation is in excess of 640 articles. Among these, the authors found 530 cases of complication of gastro-intestinal intubation of all degrees of severity beyond the trivial. This number includes a survey report of 260 cases of laryngeal and esophogeal complications with 7 deaths not further identified. Considering the tremendous number of intubation procedures used daily, the relatively few fatal cases even when other extensive complications are playing a role, and the effectiveness of surgical intervention; the surgeon armed with informed management should encounter little serious difficulty.

Rather than to list the reported complications, a few general principles may be mentioned. The possibility of complications begins with the inadequate selection, inspection and testing of the proposed tube and suction apparatus. Gentleness and thought should prevail during intubation and extubation and the duration of use especially in the very young, the aged and the debilitated. In general, laryngeal complications are the most serious and may appear late. Esophageal complications are largely associated with esophageal varices and the use of the Sengstaken-Blakemore tube. Attempts to administer a tube too rapidly may cause coiling, duplication, knot formation with resultant lack of function, pressure points and tissue neurosis. Tubes fail mechanically, may become practically or totally arrested or lost down the intestinal tract and fail to pass a variety of obstructing lesions. Tubes may pass around a Gastro-entecostomy stoma, may become associated with an intussusception, enter or extend beyond an anastomosis. While a tube using a mercury filled bag rupture can release mercury into a fistulous tract, or it can cause a pulmonary complication directly by aspiration.

Individual consideration is in order as some patients are sensitive to tube materials, and the interpretation of symptoms. As surgical management becomes more complex with the increased use of tubes entering and leaving a variety of orifices and incisions the problem of irrigating, infusing or aspirating the wrong tube is appreciable.

Finally, the history of intubation is interesting, the first attempts, the enthusiasm of the 1930's tempered by rather tardy reports of complications, and the parallel with present-day literature on substitute procedures for intubation.

Glomerulonephritis: Its Link to the Collagen Diseases (?) R. C. Muehrcke. *Postgraduate Medicine, May, 1962.*

Histopathologic and clinical features of glomerulonephritis and the collagen diseases are reviewed. While they have many facets in common, they are also dissimilar in many respects. The link of glomerulonephritis to the collagen diseases is highly questionable.

It is unwise to believe that corticosteroids can be used to suppress immunologic phenomena in a later stage of glomerulonephritis. They may have an anti-inflammatory action early in the acute stage, but their widespread use is not justified at present. In contrast, corticosteroid therapy is of specific benefit in lupus nephritis, to suppress the auto-immune phenomenon.